PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF AERIAL PARTS OF HELICANTHES ELASTICA (Decr) DANSER FOR ITS ANTIUROLITHIATIC AND NEPHROPROTECTIVE ACTIVITY

Dissertation work submitted to

The Tamilnadu Dr. M.G.R. Medical University, Chennai

In partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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EVALUATION CERTIFICATE

This is to certify that this dissertation work entitled **"PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF AERIAL PARTS OF HELICANTHES ELASTICA (Decr) DANSER FOR ITS ANTIUROLITHIATIC AND NEPHROPROTECTIVE ACTIVITY"**is the Bonafied work carried out by **NAJMUDHEEN A P, Reg. No: 261325754** under the guidance of **Prof. P. PANNEERSELVAM, M.Pharm.,** Department of Pharmacology for the partial fulfillment of the requirement of award for **Master of Pharmacy** and this is forwarded to **The Tamilnadu Dr. M.G.R Medical University, Chennai** during the academic year **2016 – 2017** has been evaluated on_____

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DECLARATION

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This is to authenticate that the plant HELICANTHES ELASTICA of family LORANTHACEAE have been collected from ABS Botanical Gardens, Kaaripatti, Salem district Tamil Nadu and handed over to NAJMUDHEEN A P is studying M.Pharm, final year in PADMAVATHI COLLEGE OF PHARMACY for his project work.

Botanically yours,

For ABS Botanical Conservation Research and Training Centre

Executive Director

Former Siddha Research Counsultant (AYUSH)

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NAJMUDHEEN.A.P

DEDICATED TO MY BELOVEDFAMILY, TEACHERS AND FRIENDS

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
ANOVA	Analysis of variance
BUN	Blood Urea Nitrogen
CDDP	Cis-di ammine dichloride platinum
Ctr 1	Copper transporter 1
CP	Cisplatin
CPCSEA	Committee for the Purpose Of Control and Supervision of experiment on animals
DAMP	Damage Associated Molecular Pattern
DNA	DeoxyRibo nucleic acid
ERK	Extracellular regulated kinase
EG	Ethylene Glycol
EAC	Ehrlich Ascites Carcinoma
GGT	Gamma Glutamyl Transpeptidase
HE	*Helicanthes elastica*
HPLC	High PerformanceLiquid Chromatography
IL	Inter Leukins
IBP	Indian Biodiversity Portal

JNK	Jun N Terminal kinase
MHE	Methanolic extract of *Helicanthes elastica*
MAP K	Mitogen Activated protein kinase
NADPH	Nicotinamide adenine dinucleotide phosphate
OECD	Organisation for Economic Co-operation and Development.
OCT	Organic Cation Transporter
PPAR	Peroxisome Proliferator Activated Receptor
RAPD	Random Amplified Polymorphic DNA
ROS	Reactive Oxygen Species
RBC	Red Blood Cell
SAPK	Stress activated protein kinase
SOD	Superr oxide dismutase
TNF	Tumour Necrotic factor
WBC	White Blood Cell

ABSTRACT

ABSTRACT

Kidney diseases are a major problem of worldwide proportion. A large number of herbs have been used traditionally to treat kidney diseases caused by various agents. The present study was a similar attempt in that direction. *Helicanthes elastica* is a less explored Indian mistletoe. Methanolic extract of *Helicanthes elastica*(MHE) was evaluated for antiurolithiatic and nephroprotective activity in Wistar rats. For antiurolithiatic study, male Wistar rats were selected and ethylene glycol (0.75%v/v) in drinking water was fed to all the groups except normal control for 28days to induce urolithiasis for preventive and curative regimen. Cystone 750mg/kg was used as the standard drug. Various renal functional and injury markers such as urine volume, calcium, magnesium, serum creatinine, serum uric acid and blood urea nitrogen were evaluated in urine and serum samples. The result indicated that administration of MHE significantly reduced and prevented the growth of urinary stones. Histopathological studies also supported the result. In nephroprotective study, Wistar rats of either sex selected and renal toxicity was induced by administering cisplatin 5mg/kg i.p on first day. From day 2 to day 14 animals of all groups except normal group were administered with MHE. Cisplatin induced renal damage is clearly evidenced by the elevated levels of serum markers like urea, creatinine and uric acid. The treatment with the extract regained the elevated levels of renal injury markers in serum to normal levels. Histopathological reports also supported the result. This study suggests that the MHE offers protection against ethylene glycol induced urolithiasis and cisplatin induced nephrotoxicity

Keywords: *Helicanthes elastica*; Urolithiasis; Ethylene glycol; Nephrotoxicity; Nephroprotection; Cisplatin; Cystone; Histopathology.

INTRODUCTION

INTRODUCTION

Medicinal plants have been known for millennia and are being used as a rich source of therapeutic agents worldwide. WHO reported that nearly 75% global population, most in the developing world depends on the botanical medicines for their basic health care needs with around 800 plants being used in indigenous systems of medicines. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines.

India is one among the world‟s 12 hot spot having the largest plant biodiversity and it has almost 45,000 plant species, of which 15,000 to 20,000 are used for medicinal purposes. Indian civilization has played a pioneer role from time immemorial in utilizing plants such as indigenous drugs and has the unique distinction of having 6 recognized systems of medicine in this category. They are Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homeopathy.A large no of healers in the folklore stream is another system that has not been recognized under any category.[1,2]The major classical system of medicines used in Ayurveda, Siddha and Unani together use about 1200 plant species to treat human ailments whereas the tribes of India use more than 7500 plant species. Ethno medicinal practices are the mother of all other traditional systems. Traditional medicine is the sum total of knowledge skills and practices based on the theories, beliefs and experiences indigenous to different cultures. Whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnes. Traditional folk medicine serves as the root of Ayurveda. [2]Historically and culturally, oral folk traditions have had a symbiotic relationship with codified Indian medical systems like Ayurveda, Siddha, Unani and Swarigpa. The medical research and commerce exploited many valuable experiences from the oral health traditions.WHO estimated that 80-90% of the world‟s population relies mainly on the local herbal practitioners. Various studies revealed that about 80% of the population of developing countries partially or fully depends on herbal drugs for primary health care. Traditional medicine relies on higher plants as the main source of drug therapy. Herbal medicine is not just a poor man‟s substitute for conventional medicine but a valuable form of treatment in its own right. The

rising cost of the prescription drugs which are being used in the maintenance of normal/ personal health and wellbeing fuelled the reliability of man‟s interest in medicinal plants as a remerging health aid.

Human body, for its normal functioning deals with hundreds of thousands of independent factors which involves interactions of a large no of proteins and cells working with environmental factors. Even a small interruption in this interaction can cause a state of discomfort in which the normal functioning of the body is affected which is generally termed as a disease. Kidney, an important excretory organ in the human body function not only to excrete the metabolic waste products, but also to maintain the acid base balance and endocrine functions like erythropoietin production. Urolithiasis and nephrotoxicity are two common problems faced by the people in the current days.

Urinary stone disease has been a challenge for the humankind since a long time and can lead to serious health consequences for patients throughout their life. Urolithiasis (urinary calculi or stone) , one of the most common urinary tract disease with worldwide prevalence and incidence refers to the calcification that form in the urinary system, primarily in the kidney referred to as nephrolithiasis or ureter as ureterolithiasis and may also form in or migrate into the lower urinary system either in the bladder or urethra. The etiology behind the disorder is multifactorial and the lifestyle habits or practices strongly contribute to it. Diet and lifestyle are the two major factors thought to influence the susceptibility to many diseases.[3] Urolithiasis can be classified under a life style disease. Any factor that reduces the urinary flow or causes obstruction which results in urinary stasis or reduces urinary volume through dehydration and inadequate fluid intake, increase the risk of developing stones.[4]Thus urolithiasis is a complex process that results from several physicochemical events including crystal nucleation, aggregation and growth of insoluble particles in the kidney.

Urinary stones have been documented historically as far as back as the Egyptian mummy. The archaeological findings give profound evidence that for centuries human have been suffering from kidney and bladder stones. [5]The term urolithiasis is originated from the French word “urine” which in turn, stems

from the Latin term “urina” and the Greek “Ouron” meaning urine .The stones themselves are called as renal calculi. The word “calculus”(plural calculi) is the Latin word for pebble.

Urolithiasis is a worldwide problem. It is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70-80% in men and 47-60 in women and in economically developed countries the prevalence rate ranged between 4% and 20%.[6, 7]Women have a bio modal age of onset, with episodes peaking at 35 and 55 years. Without preventive treatment the recurrence rate of calcium oxalate calculi increases with time and reaches 50% at 10 years. [8]

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, NSAID‟s, chemotherapeutic agents have been added to the therapeutic arsenal in recent years. Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Early literatures have prescribed various herbs for the cure of renal disorders. Co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents may attenuate its toxicity. The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. In addition to this, there is a failure of regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure.

Even though kidney stone and nephrotoxicity was categorised among one of the most prevalent and widespread disease in the world, till date no guaranteed cure is found for the same. None of the known and available treatments prevent the reoccurrence of kidney stone formation and toxicity produced by cisplatin. Hence the dire need for the herbal formulation appears to be the need of the hour. The present study was carried out to evaluate the anti urolithiatic and nephroprotective activity of the methanolic extract of the whole plant *Helicanthes elastica.*

AIM AND OBJECTIvE

AIM & OBJECTIVE

AIM

To evaluate the Antiurolithiatic and Nephroprotective activity of the methanolic extract of aerial parts of *Helicanthes elastica* (Desr.) Danser.

OBJECTIVES

Phase **1**

- Literature survey

Phase **2**

- Identification and authentication of *Helicanthes elastica.*
- Collection, shade drying and powdering of leaves.
- Preparation of the methanol (80%) extract by Soxhlet extraction.
- Preliminary phytochemical screening to investigate the chemical composition.

Phase **3**

- Submission of application to IAEC.

Phase **4**

- To evaluate the Antiurolithiatic activity

  Ethylene glycol induced urolithiasis.
- To evaluate the nephroprotective activity

  Cisplatin induced nephrotoxicity

Phase **5**

- Statistical evaluation- ANOVA and scientific documentation.

REVIEW OF LITERATURE and background

REVIEW OF LITERATURE AND BACKGROUND

PLANT PROFILE

Mistletoe is the common name for the most obligate hemi parasite plants in the order Santalales. They attach to and penetrate the branches of a tree or shrub by a structure called haustorium through which they absorb water and nutrients from the host plant. In India 6 mistletoes are considered to have medicinal properties in which two belongs to genus *Loranthus* and four to the genus *Viscum. Helicanthes elastica* (Desr.)Danser belonging to the family Loranthaceae is a less known Indian mistletoe growing commonly on mango tree (*Mangifera indica*) as hemiparasites.

Fig 1.*Helicanthes elastica* growing on mango trees

Synonyms

Helicanthus elastica (Desr.)Danser

Dendrophthoe elastica(Desr.) Danser

Loranthus elasticus Desr.

Loranthus euphorbiae Wight

IBP Taxonomy Hierarchy

Kingdom: Plantae Phylum

: Tracheophyta Class :

Magnoliopsida Order :

Santalales Family :

Loranthaceae Genus :

Helicanthes

Species : *Helicanthes elastica*

Description [9]

It is a hemi parasitic dichotomously branched, glabrous; pendulous shrub with swollen joints, the young branches being green and flowers white in colour.

Leaves: opposite 4-8 X 1.5-4cm, ovate or elliptic oblong, base: - obtuse or truncate, apex:-acute or obtuse, thickly coriaceous, glaucous beneath, basically three nerved sessile or sub sessile.

Flowers: aggregated in short axillary fascicles.

Flower colour: green, white

Calyx: minute, flask shaped, margin truncate.

Corolla: white with green stripes, 2.5- 3.5cm long, split lengthwise into 5 linear, twisted lobes.

Stamen: five exserted, filament crimson.

Ovary: 1.5mm long

Style: 3-3.5cm long

Stigma: ovoid

Berry: 6-8 x 3- 3.5mm, obovoid red.

Literature review of plant *Helicanthes elastica*

Stem parasite: yes

Flowering and fruiting: December –March

Habitat: Evergreen and moist deciduous forest, also in the plains

Distribution: mainly in Western Ghats

Altitude: 600-750

Uses

Traditional use: The leaves of *Helicanthes elastica* were used to check abortion . It is also used in vesical calculi and kidney infections. Leaves are used as poultices in sores and ulcers.

Pharmacologically reported activities:-

Antimicrobial activity [10]

Antioxidant activity [11]

Antihyperglycemic activity [12]

Hepatoprotective activity [13]

Diuretic and natriuretic activity [14]

Chemical Constituents

The whole plant of *Helicanthes elastica* is found to contain sterols, terpenoids, flavones, tannins and glycosides. Alkaloids, Quinone‟s and Coumarins were absent.

The presence of compounds viz,1-octadecene,neophytadeine,hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, stigmasterol, γ-stigmasterol, β-stigmasterol-3-ol, pentacosane and -sitost-4-en-3-one by GC-MS analysis were also reported. The column chromatography of the ethyl acetate extract also showed the presence of friedelin, epifriedelinol, β amyrin, β sitosterol, ethyl

gallate, gallic acid and β sitosterol 3-β-D glucopyranoside.[15, 16, 17]

Sunil et al., (2015) evaluated the phytoconstituents of *Helicanthus elastica* whole plant. The presence of compounds viz,1-octadecene, neophytadeine, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, stigmasterol, γ-stigmasterol, β-stigmasterol-3-ol, pentacosane and -sitost-4-en-3-one were detected by GC-MS analysis and 7 compounds viz, friedelin, epifriedelinol, β amyrin, β sitosterol, ethyl gallate, gallic acid and β sitosterol 3-β-D glucopyranoside by column chromatography were reported.[15]

Sunil et al., (2014) reported the antimicrobial potential of *Helicanthus elastica* (Desr.) Danser (Loranthaceae). The zone of inhibition and minimum inhibitory concentration were assayed by standard methodologies against bacteria like *Aeromonas hydrophila, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Vibrio fischeri, and a fungus Candida albicans*. The study concluded that, of the eight tested bacteria, the alcoholic extract of *H. elastica* was found to be active against *K. pneumoniae*, A. hydrophila, *E. coli*, and *V. fischeri* at concentration ranging from 250 to 500μg/ml and *C. albicans* showed inhibition only at a concentration of 2000μg/ml.[10]

Sunil et al., (2016) reported the hepatoprotective activity of *Helicanthus elastica*. The acute oral toxicity study conducted on the aqueous and 95% ethanolic extract of whole plant of *Helicanthus elastica* revealed that the extract is safe up to a dose of 2000 mg/kg body. The hepato protective activity of the extract(200 and 400mg/kg body weight) against paracetamol induced toxicity was evidenced by significant reduction in the elevated serum transaminase levels and the histopathological studies that showed marked reduction in the fatty degeneration and centrizonal necrosis, in animals receiving different doses of extract along with paracetamol as compared to the control group. [13]

Sunil et al., (2013) developed the finger print profile of chloroform and ethanolic extracts of *Helicanthus elastica*. The presence of different marker

compounds was reported by fingerprinting the extract in suitable solvent system and by scanning densitometrically under UV and after dipping in vanillin-sulphuric acid reagent followed by heating at 105°C. The study revealed differentiating fingerprints which are helpful in the authentication of *H. elastica*.[16]

Sunil et al., (2014) reported the total phenol content and antioxidant properties of the ethanolic extract of *Helicanthus elastica* using different *in vitro* models. The total phenol content, reducing power assay and scavenging of free radicals like nitric oxide, hydroxyl, hydrogen peroxide, and 1,1-diphenyl-2-picrylhydrazyl were evaluated by standardized *in vitro* chemical methods using ascorbic acid as the standard and reported that the total phenol content of the plant to be 1.89% w/w. The study also reported that the extract showed good reducing power as well as scavenging of free radicals (nitric oxide, hydroxyl, superoxide anion, and hydrogen peroxide) at concentrations ranging from 5 to 100µg/ml. [11]

Sunil et al., (2015) reported the molecular differences in the genomic DNA of the plant while growing on five different host trees using four random markers employing random amplified polymorphic DNA (RAPD) followed by similarity matrix by Jaccard‟s coefficient and distance matrix by hierarchal clustering analysis. The study reported that the DNA of *H. elastica* differs depending upon the host on which it grows, hence the host must be considered while studying or utilizing this mistletoe for medicinal purposes.[17]

Sunil et al., (2014) studied the whole plant of *Helicanathus elastica* for physicochemical and nutraceutical analysis aiding standard methodology. They fingerprinted the total ethanolic extract with HPLC-UV. Under the head physicochemical examination, determined various parameters like moisture content, total ash, water-soluble ash, acid-insoluble ash, alcohol-soluble and water-soluble extractive, successive extractive values by cold and hot extraction, heavy metals like arsenic, lead, cadmium and mercury, total bacterial count, total fungal count, presence of enterobacteriaceae, *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa* and *Staphylococcus aureus* and

presence of carbohydrate, fat, protein and fibre**,** calorific value, vitamins like niacinamide, pyridoxine, riboflavin, thiamine and ascorbic acid, trace elements like iron and zinc. The investigations revealed the presence of appreciable quantity of important vitamins and trace elements in the plant.[18]

Rajesh et al., (2015) evaluated the anti-hyperglycaemic activity of methanolic extract of *Helicanthus elasticus* on Streptozotocin induced diabetic rats. The activity was confirmed by observing the significant reduction in the blood glucose level at 0, 30, 60,120 and 240 min in fasted and glucose loaded diabetic rats in acute studies and at 1^{st}, 7^{th}, 14^{th} and 21^{st} day in sub-acute studies of Streptozotocin induced diabetic rats after extract administration at 200 mg/kg body weight. Thus they concluded that in both acute and sub-acute studies, the methanolic extract has shown statistically significant antihyperglycemic and glucose tolerance activity.[12]

Shihab et al., (2006) studied the antioxidant, antinociceptive, and general toxicity of the ethanolic extract of the leaves of *H.elastica*. The extract showed potent antioxidant activity (IC50 5.1 μg/ml) using DPPH radical scavenging assay, which is comparable to the standard ascorbic acid (IC50 4.6 μg/ml). They also reported that the extract significantly and dose dependently inhibited the acetic acid induced writhing in mice (71.2%, $P < 0.001$ and 28.0%, $P < 0.05$ for 500 and 250 mg/kg body weight, respectively). Using different chromatographic techniques, quercitrin (quercetin 3-O-α-rhamnoside) was separated as the major component from the extract and elucidated the structure by detailed 1D and 2D NMR and mass spectral analysis.[19]

Namita et al., (2010) reported the diuretic activity of methanolic extracts of *Viscum articulatum* (VA) Burm. f. and *Helicanthus elastica* (HE) (Ders.) Dans. in rats. Diuretic, saluretic and natriuretic effects were reported after the oral administration of the extracts. The polyphenolic and triterpenoid contents were determined quantitatively using chemical assays and high performance liquid chromatography (HPLC) analysis respectively. Phytochemical analysis revealed the presence of polyphenolics and triterpenoids, such as oleanolic acid and lupeol, as the major phytochemicals involved. A higher content of

polyphenolics in association with lower triterpenoid content was found to favour potassium-sparing effects.[14]

KedarKalyani et al., (2012) Studied the triterpenoid pattern of cuticular wax of leaves of *Helicanthus elasticus* Linn. (Loranthaceae) parasitic on *Memecylonum bellatum* Burm.f. (Melastomataceae) and compared with standard triterpenoids such as lupeol, olenolic acid and betulin. The cuticular extract was also subjected to column chromatography and characterised the isolated compounds by UV, IR, HPTLC and MS studies and compared with standards. From the results they concluded that the cuticular wax of *Helicanthus elasticus* Linn shows presence of lupeol.[20]

Nipun et al., (2011) explored the anticancer activity of the ethanolic and aqueous extracts of the *Dendrophthoe falcate* in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line. The significant decrease ($p<0.0001$) in the tumor volume, viable cell count, tumor weight and elevated life span of EAC tumor bearing mice after the administration of ethanolic extract (200 and 400 mg/kg body) for 13 consecutive days were considered as the parameter to confirm the anticancer ativity . Haematological profiles such as red blood cell (RBC), haemoglobin, and white blood cell (WBC) count reverted to normal level in treated mice. The results demonstrated that the extract has potent dose dependent anticancer activity comparable to that of cisplatin. [21]

Pattanayak et al., (2008) Investigated the ethanolic extract of aerial parts of *Dendrophthoe falcata* for the evaluation of its healing efficiency on excision and incision wound models in rats. The results showed that *Dendrophthoefalcata* extract has potent wound healing capacity as evident from the wound contraction and increased tensile strength. Correlation of Hydroxyproline and hexosamine expressions with the healing pattern was also reported. The antimicrobial activity of various fractions of extract (petroleum ether, chloroform and ethanol) were also reported by screening against the organisms: *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeroginosa*, *Serratia*

marcescens, and five fungi *Candida albicans, Candida tropicalis*: dimorphic fungi, *Aspergillus fumigatus, Aspergillus niger*: systemic fungi, and some infectious bacteria *Escherichia coli*, and *Salmonella typhi* and has reported a positive result.[22]

Osadebe et al., (2014) reported the hypoglycaemic and anti-hyperglycemic activities of dried leaves of *Loranthusmicranthus* (Linn.) (Loranthaceae), parasitic on *Persea americana, Baphianitda*, *Kola acuminata, Pentaclethra macrophylla, Azadirchta indica,* in normoglycemic and alloxan-induced diabetic albino rats. Intraperitoneal administration with 200 mg/kg of the respective methanolic extracts of *Loranthus micranthus* (Linn.), glibenclamide (positive control), and 20% (v/v) Tween 20 solution (negative control) showed significant reduction in the sugar levels of the withdrawn blood samples in Normoglycemic and alloxan-induced diabetic rats .[23]

KIDNEY

The paired kidneys are reddish, kidney-bean-shaped organs located just above the waist between the peritoneum and the posterior wall of the abdomen. The kidneys are located between the levels of the last thoracic and third lumbar vertebrae. The liver occupies considerable space on the right side superior to the kidney.

Anatomy of the kidneys

An adult kidney is 10-12cm long, 5-7 cm wide and 3 cm thick. The concave medial border of each kidney faces the vertebral column. Three layer of tissue surround each kidney. The deep layer, the renal capsule is a smooth, transparent sheet of dense irregular connective tissue. The middle layer, the adipose capsule is a mass of fatty tissue surrounding the renal capsule. The superficial layer, the renal fascia is another layer of dense irregular connective tissue.

The frontal section through the kidney reveals two distinct regions. The superficial region is called renal cortex, a smooth-textured reddish area. The deep reddish-brown inner region is called the renal medulla. It consists of 8-18 cone-shaped renal pyramids.

Together, the renal cortex and renal pyramids of the renal medulla constitute the parenchyma of the kidney. Within the parenchyma about one million microscopic structures are present called nephrons. Nephrons are the functional units of the kidney. Each nephron consists of two parts, a renal corpuscle, where blood plasma is filtered and renal tubule into which the filtered fluid passes.

Blood supply to the kidneys is provided by renal artery that rises from the aorta. Venous tributaries unite to form the single wide renal veins which drain into the inferior vena cava. Nerve supply is provided by sympathetic nerves originated in the 12th thoracic and 1st lumbar segments. The parasympathetic nerves are derived from the vagi. The frontal section of the right kidney is shown in figure 2.

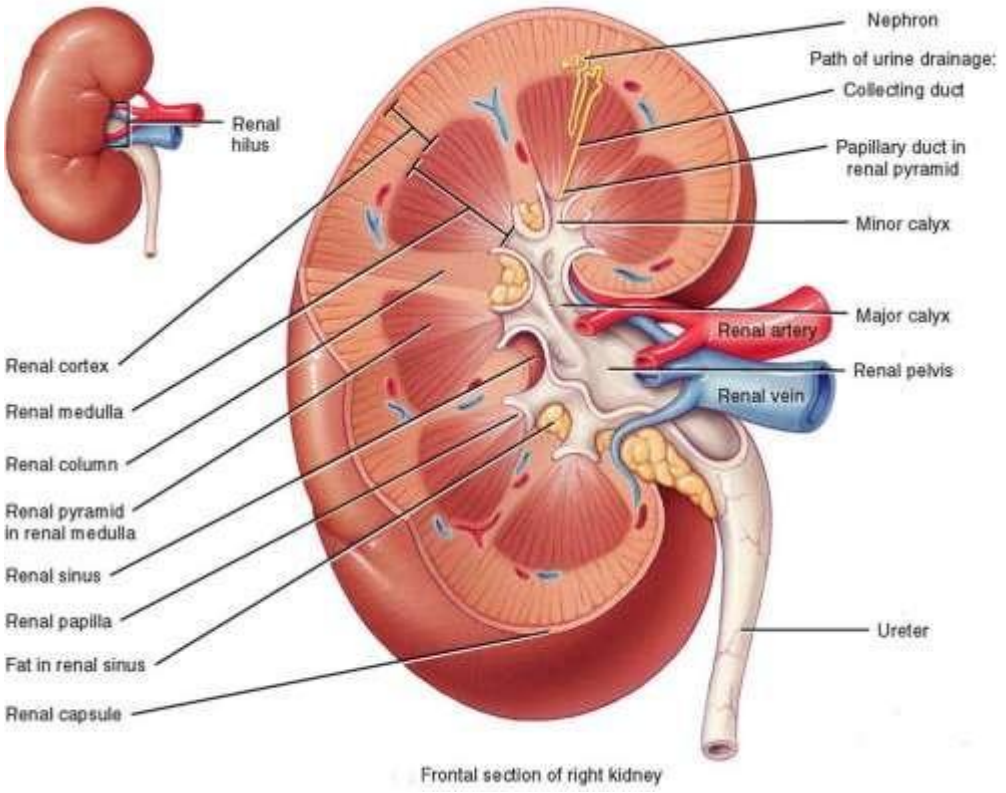


Figure 2: Frontal section of the right kidney

Nephrons

Nephrons are the structural and functional units of the kidney. Each nephron consists of a glomerulus, proximal tubule-comprising convoluted and straight segments, loop of Henle, distal convoluted tubule and collecting duct. The glomerulus comprises a tuft of capillaries projecting into a dilated end of the

renal tubule. Most nephrons lie largely or entirely in the cortex. The remaining 12%, called the juxtamedullary nephrons, have their glomeruli and convoluted tubules next to the junction of the medulla and cortex, and their loops of Henle pass deep into the medulla. In juxtamedullary nephrons, part of the thick ascending limb of the loop of Henle (as well as the thin part of the loop) lies in the medulla; the part of the medulla containing the thick ascending limb is known as the outer stripe of the medulla, as opposed to the inner stripe, which contains thin segments only.

Histology of the kidney

Each kidney consists of a medulla and a cortex which is both outside and between the pyramids. In the latter situation the cortex forms the renal columns through which pass the renal vessels. The histological and functional unit of the kidney is nephron (renal tubule) composed of a glomerular (Bowman‟s) capsule, a proximal convoluted tubule, a loop of Henle, a distal convoluted tubule and a collecting duct.

The cup-like glomerular capsule lies in the cortex around a tuft of capillaries, the glomerulus, and leads into the proximal convoluted tubule. The loop of Henle descends into the medulla nearly as far as the apex of the pyramid and then returns to the cortex and continues as the distal convoluted tubule. The distal tubules join the collecting ducts which pass through the medulla and open on the surface of a renal papilla into a minor calyx. The glomerular capsule is lined by thin specialized epithelium, cubical epithelium lines. The proximal and distal convoluted tubules are adjacent parts of the loop of Henle. The rest of the loop is lined by simple squamous and the collecting ducts by columnar epithelium.

Functions of the kidneys

a) **Formation of urine.**

The production of urine is vital to the health of the body. Most of us have probably never thought of urine as valuable, but we could not survive if we did not produce it and eliminate it. The composition of urine reflects the activities of the nephrons in the maintenance of homeostasis. Waste products of protein metabolism are excreted, electrolyte balance is maintained and the acid-base

balance is influenced by the excretion of hydrogen ions. To produce urine, nephrons and collecting ducts perform three basic processes.

• Glomerular filtration

• Tubular reabsorption

• Tubular secretion

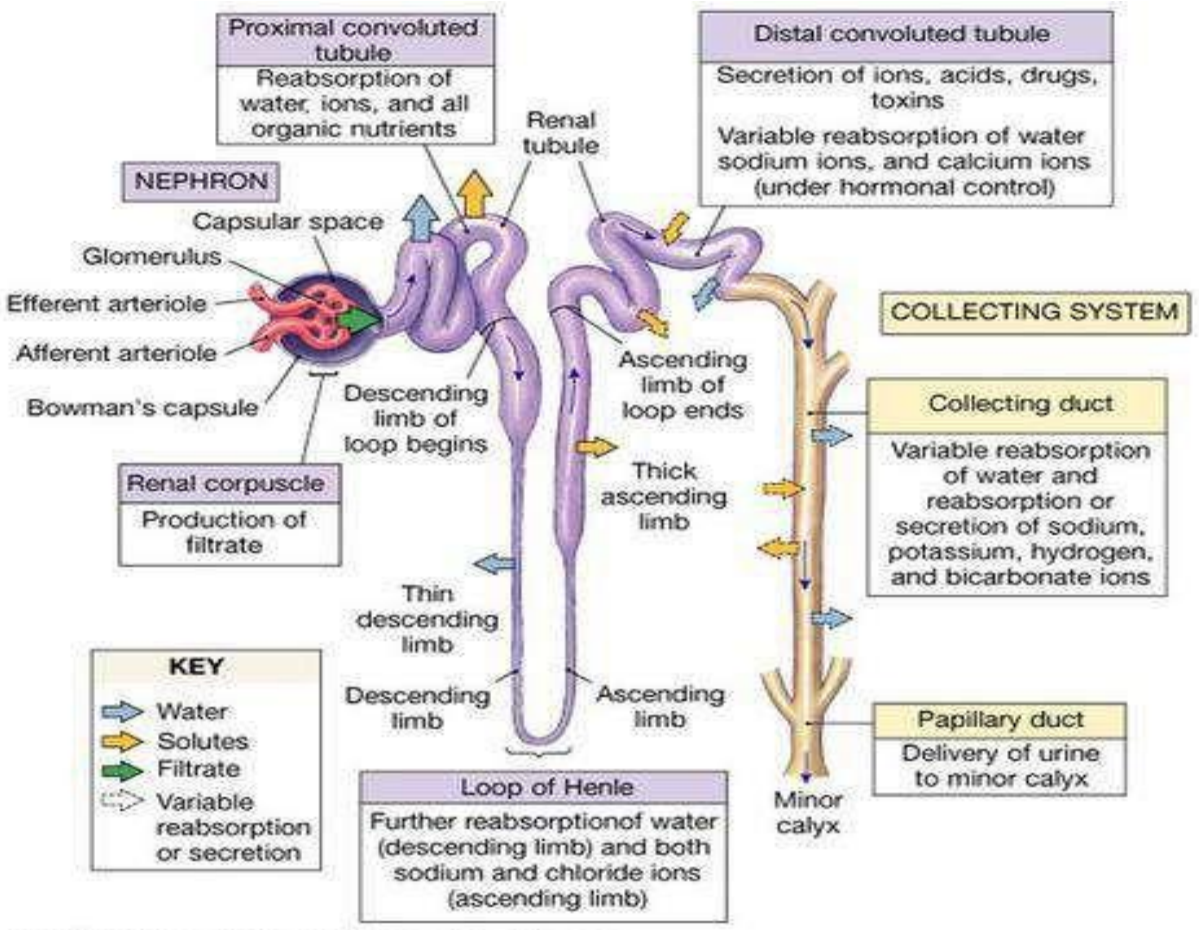


Figure3 : Urine formation

b) Regulation of blood ionic composition.

The kidneys help regulate the blood levels of several ions, most importantly sodium ions (Na^+), potassium ions (K^+), calcium ions (Ca^{++}), chloride ions (Cl^-) and phosphate ions (HPO_4^{2-}).

c) Regulation of blood pH.

The kidneys excrete a variable amount of hydrogen ions (H^+) into the urine and conserve bicarbonate ions (HCO_3^-), which are an important buffer of H^+ in the blood. Both of these activities help to regulate blood pH.

d)Regulation of blood volume.

The kidneys adjust blood volume by conserving or eliminating water in the urine.

e) Regulation of blood pressure.

The kidneys help to regulate blood pressure by secreting the enzyme renin**,** which activates the renin-angiotensin-aldosterone pathway. Increased renin causes an increase in blood pressure.

f) Maintenance of blood osmolarity.

By separately regulating loss of water and loss of solutes in the urine the kidneys maintain relatively constant blood osmolarity close to 300 milliosmoles per liter.

g) Production of hormones.

The kidneys produce two hormones. Calcitriol, the active form of vitamin D, helps to regulate calcium homeostasis and erythropoietin stimulates production of red blood cells.

h) Regulation of blood glucose level.

The kidneys can use amino acid glutamine in gluconeogenesis, the synthesis of glucose molecule. Then they release glucose into blood to maintain normal glucose level.

i)Excretion of wastes and foreign substances

By forming urine, kidneys help to excrete wastes (substances that have no useful function in the body)

UROLITHIASIS

The formation of stone in the urinary system, i.e. in the kidney, ureter, and urinary bladder or in the urethra is called urolithiasis. „Urolithiasis" = ouron (urine) and lithos (stone). Urolithiasis is one of the major diseases of the urinary

tract and is a major source of morbidity. Stone formation is one of the painful urologic disorders that occur in approximately 12% of the global population and its re-occurrence rate in males is 70-81% and 47-60% in female. It is assessed that at least 10% of the population in industrialized part of the world are suffering with the problem of urinary stone. It has been found that the formation of urinary calculi dates back not only to 4000 B.C in the tombs of Egyptian mummies also in graves of North American Indians from 1500 to1000 B.C. Stone formation is also documented in the early Sanskrit documents during 3000 and 2000 B.C. The problem of stone formation is considered as a medical challenge due to its multifactorial etiology and high rate of reoccurrence. Stone formation is also caused due to imbalance between promoters and inhibitors.

CLASSIFICATION OF STONES

The crystalline mineral deposits that form in the kidney constitute the renal calculi. They develop as microscopic crystals in the Loop of Henle, the distal tubule, or the collecting duct and they can enlarge to form visible fragments.A large number of factors contribute to the formation of calculi and are formed by different mechanisms. The process of stone formation depends on urinary volume, concentration of calcium, phosphate, oxalate, sodium and uric acid ions; concentration of natural calcium inhibitors like citrate, magnesium, bikunin, etc. and the urinary pH[26]High ion levels, low urinary volume, low pH. and low citrate levels also favours calculi formation.

The most common type of stones occurring were as follows

Table 1: Common types of stones

Name of stone	Constituents	Incidence
Calcium oxalate	Calcium, oxalate	34%
Calcium phosphate	Calcium, phosphate	33%
Uric acid	Uric acid	8%
Struvite	Ammonium,magnesium,phosphate	15%
Cystine	Cystine	3%
Medication induced stones	Depends on the medication (eg; Indinavir, ephedrine, Guaifensin, silica etc)	1%

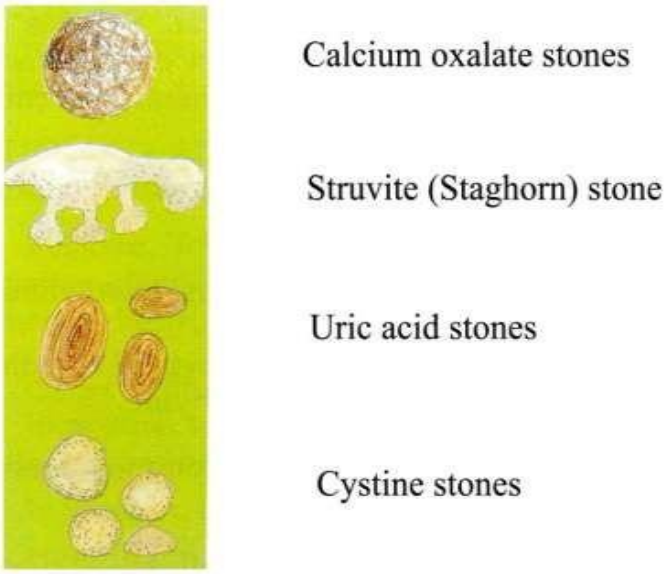


Figure 4: Different types of Stones

Calcium oxalate stones

- Most common type and represent about 80%
- Contains calcium oxalate alone or in combination with calcium phosphate in the form of apatite or brushite
- They appear as 'envelopes' microscopically. They may also form 'dumbbells"
- Hydrated forms of the compound occur naturally as three mineral species: whewellite (monohydrate, known from some coal beds), weddellite (dihydrate) and a very rare trihydrate called caoxite. [27]
- Formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and renal tubular acidosis.
- Oxaluria is increased in patients with certain gastrointestinal disorders including inflammatory bowel disease such as Crohn disease or patients who have undergone resection of the small bowel or small bowel bypass procedures and in patients who consume increased amounts of oxalate (found in vegetables and nuts). Primary hyperoxaluria is a rare autosomal recessive condition which usually present in childhood. [28]

Struvite stones (Ammonium magnesium phosphate, $NH_4MgPO_4 \bullet 6H_2O$)

- Constitute about 10-15% of urinary calculi.
- Also known as infection stones
- Magnesium, ammonium and phosphorus are the building blocks for the formation of Struvite crystals in urine
- They have „coffin lid' morphology by microscopy.
- They form most often in the presence of infection by urea-splitting bacteria.
- Using the enzyme urease, these organisms metabolize urea into ammonia and carbon dioxide which alkalinizes the urine, resulting in favourable conditions for the formation of struvite stones.
- In addition, urine pH and its influence on the concentration of trivalent ionic phosphate (PO_4^{3-}) play a key role in struvite crystallization. As urine pH increases, H_3PO_4, $H_2PO_4^{1-}$and $HPO4^{2-}$are rapidly deprotonated

(i.e., removal of hydrogen ions) increasing the concentration of PO_4^{3-} a principal component and driving force for struvite crystal formation.

- Commonly observed in people who have factors that predispose them to urinary tract infections, such as those with spinal cord injury and other forms of neurogenic bladder, ileal conduit urinary diversion, vesico ureteral reflux, and obstructive uropathies and in people with underlying metabolic disorders, such as idiopathic hypercalciuria, hyperparathyroidism, and gout. Infection stones can grow rapidly, forming large calycealstaghorn (antler-shaped) calculi requiring invasive surgery such as percutaneous nephrolithotomy for definitive treatment.[29]

Uric acid stones

- About 5–10% of all stones are formed from uric acid.
- They appear as pleomorphic crystals, usually diamond-shaped. They may also look like squares or rods which are polarizable. [28]
- People with certain metabolic abnormalities; including obesity may produce uric acid stones. They also may form in association with conditions that cause hyperuricosuria (an excessive amount of uric acid in the urine) with or without hyperuricemia (an excessive amount of uric acid in the serum) and in association with disorders of acid/base metabolism where the urine is excessively acidic (low pH), resulting in precipitation of uric acid crystals. [30]

Cystine stones

- Constitutes about 3% of the urinary calculi.
- Mainly caused due to cystinuria, an inherited (genetic) disorder of the transport of an amino acid (a building block of protein) called cystine that results in an excess of cystine in the urine (cystinuria) and the formation of cystine stones.
- Although cystine is not the only overly excreted amino acid in cystinuria, it is the least soluble of all naturally occurring amino acids.
- Cystine tends to precipitate out of urine and form stones (calculi) in the urinary tract.

- Small stones are passed in the urine. However, big stones remain in the kidney (nephrolithiasis) impairing the outflow of urine while medium-size stones make their way from the kidney into the ureter and lodge there further blocking the flow of urine (urinary obstruction).
- Obstruction of the urinary tract puts pressure back up on the ureter and kidney causing the ureter to widen (dilate) and the kidney to be compressed. Obstruction also causes the urine to be stagnant (not moving), an open invitation to repeated urinary tract infection. The pressure on the kidneys and the urinary infections results in damage to the kidneys. The damage can progress to renal insufficiency and end-stage kidney disease, requiring renal dialysis or a transplant.[31]

Silicate stones or drug induced stones

- Very rarely observed
- Formed as a result of taking certain medications or herbal products and the subsequent build-up of chemicals from those products in the urine. Some of these are Loop diuretics, Acetazolamide, Topiramate, Zonisamide, Laxatives (when abused), Ciprofloxacin, Sulfamedications,Triamterene, Indinavir, Ephedrine, Guaifenesin, and products containing silica.

ETIOLOGY OF STONE FORMATION

Stone formation is usually multi-factorial with more than one element increasing a patient,,s risk for stone formation and is strongly related to dietary lifestyle habits or practices. Increased rates of hypertension and obesity, which are linked to nephrolithiasis, also contribute to an increase in stone formation.[32]About 25% of patients with urolithiasis may be the result of a polygenic defect with partial penetrance. Several disorders that cause renal stones are hereditary and they are renal tubular Acidosis and Cystinuria.[33]Xanthinuria and dihydroxyadeniuria are rare hereditary disorders.

A slightly higher rate of renal stone disease is reported in males than in females. Men are affected approximately 4 times more often than women. The rate of

occurrence is three times higher in men than women, because of enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation. The prevalence of urinary calculi is higher in mountainous, desert or tropical areas. Other high incidence areas are the British Isles, Scandinavian countries, Mediterranean countries, Northern India and Pakistan, Northern Australia, Central Europe, portions of the Malayan Peninsula and China

Increased water intake and increased urinary output decrease the incidence of urinary calculi in those patients who are predisposed to the disease. The presence or absence of certain trace elements in water has been implicated in the formation of urinary calculi. For example, zinc is an inhibitor of calcium crystallization. Dietary intake of various foods and fluids that result in greater urinary excretion of substances that produce stones has a significant effect on incidence of urinary calculi. Ingestion of excessive amounts of purines oxalates, calcium, phosphate, sodium and other elements often results in

excessive excretion of these components in urine.[34]There is a relationship between occupation, social class and risk of stone formation. The risk of formation of urinary calculi was increased in the most affluent countries, regions, societies and individuals. These inhabitants have more disposable income to spend on animal protein, which leads to increased urinary concentrations of calcium, oxalate and uric acid.[35]

Table no 2: Major causes of calcium stone formation

Condition	Definition	Cause
Hypercalciuria	Urinary calcium excretion >200 mg/dl	**Absorptive hypercalciuria:** ↑GI calcium absorption renal. **hypercalciuria:** impaired renal Calcium absorption **Resorptivehypercalciuria:** Primary hyperparathyroidism.
Hyperoxaluria	Urinary oxalate excretion > 40mg/dl	**Primary hyperoxaluria:** genetic Oxalate overproduction dietary **Hyperoxaluria:** excessive dietary intake **Enteric hyperoxaluria:** ↑GI oxalate absorption
Hypocitraturia	Urinary citrate excretion < 320 mg/dl	**Distal renal tubular acidosis:** impaired renal tubular acid Excretion. **Chronic diarrhea syndrome:** GI alkali loss **Thiazide-induced:** hypokalemia **Idiopathic hypocitraturia:** High animal protein diet, excessive physical exercise, high sodium intake.

Hyperu ricosuria	Urinary acid excretion > 600 mg/dl	Dietary urine excess, uric acid overproduction or over excretion
Hypomagnesuria	Urinary magnesium excretion < 50mg/dl	Limited intake of magnesium-rich foods
Gouty diathesis	urinary pH < 5.5	Etiology unknown

PATHOPHYSIOLOGY OF UROLITHIASIS

A number of crystalloids of different types (oxalate, uric acid, calcium, cystine etc) are present in the urine which is kept in the solution by means of colloids like mucin and sulphuric acid by the process of absorption.

When an imbalance arises in the crystalloid-colloid ratio, i.e., an increase in crystalloid and fall in colloid level occurs, the crystalloids begins to precipitate and adhere to the cells on the surface of renal papilla, a seed crystal can grow and aggregate into an organized mass. Depending on the chemical composition of the crystal, the stone-forming process may precede more rapidly when the urine pH is unusually high or low. [27]Thus, the renal stones or when the colloid loses the solvent action or adhesive property, urinary stones are formed.

Urolithiasis/Nephrolithiasis results in increased oxalate (hyperoxaluria), calcium, phosphate, uric acid and cysteine in the urine. Hyperoxaluria being a major risk in urolithiasis leads to the activation of renin angiotensin which in turn increases the angiotensin II levels. Angiotensin II causes the activation of NADPH oxidase and as a result reactive oxygen species are generated which causes lipid peroxidation in the renal cell membrane. The inflammation resulted from the lipid peroxidation provides sites for the crystal attachment and the crystal so formed induces renal colic pain and chances for infections.[36]

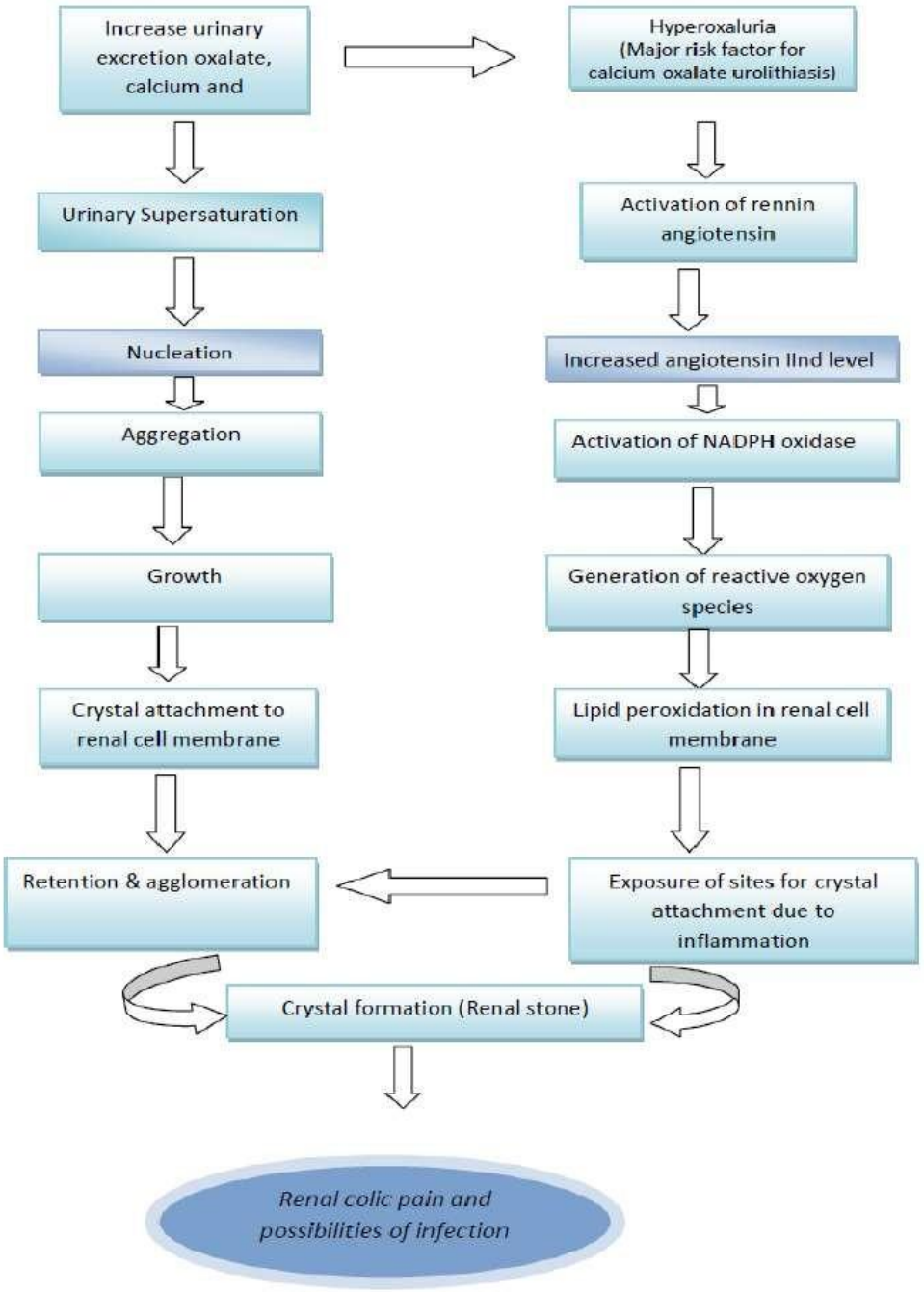


Figure 5: Pathophysiology of renal stone formation

SYMPTOMS OF KIDNEY STONE [37]

Kidney stones can cause intense pain and blockage as they pass downwards. Any or some of the following symptoms are noticed:

- Severe loin pain that comes and goes in the back or side between the pelvis and lower rib. This is known as renal colic. The pain can move down into the groin or genitals
- Nausea and vomiting
- Difficulty in passing urine
- Visible or microscopic traces of blood in the urine
- Sweating, fever and shivers.

Food and beverages that enhance the risk of calculi formation [38]

- Foods rich in organic acids (oxalates) like spinach, rhubarb, nuts and wheatbran.
- Cranberry juice, apple juice, Grape fruit juice and dark colas.
- Animal protein rich foods like meat, eggs and fish.
- Supplements like vitamin C & D.
- High intake of salt.
- Alcohol.

Foods and beverages that control the risk of calculi formation

- Foods such as radish, beet root and horsegram.
- Citrus and lemon juices, coffee, tea and soft drinks rich in citrates.
- Low protein diets and fibre rich foods.
- Supplements like Vitamin E, B6, and magnesium.
- Low salt diets.
- Beer and wine to a little extent as they contain purines.

TREATMENT OF KIDNEY STONES

Management of any stone is dictated by stone location, composition, size and patient factors e.g. co-morbidities, solitary kidney and occupation. All management of stones can be thought of as acute, definitive and preventative. Preventative management involves education of patients to decrease their risk of stone disease by modifying diet and hydration. Hydration is the most vital step in prevention as chronic dehydration has been identified as a cause of urolithiasis. Increasing fluid intake has been shown to decrease stone incidence. in calcium oxalate calculus formers that increasing the urine output to greater than 2 litres a day, results in a 12% recurrence in stone formation, compared to

those with no specific fluid recommendations who had a 27% recurrence of calculi . The goals of treatment are to control symptoms, render the patient stone free and prevent recurrence.

Many allopathic agents like Thiazide diuretics (e.g. Hydrochlorothiazide), Alkali (e.g. Potassium citrate), Allopurinol, Sodium cellulose phosphate (SCP), Penicillamine (Cuprimine), Analgesic (Diclophenac sodium), Bisphosphonates, Potassium phosphate, OxalobacterFormigenes and other probiotics are used in treating the stones formed. The ayurvedic medicines used in the treatment are Cystone, Calcuri, Chandraprabhabati, Trinapanchamool, Rencare Capsule, Patherina tablet, Ber Patthar Bhasma, Chander Prabhavati.

Surgical Method[39]

Currently there are four methods for stone removal

a) Extracorporeal shockwave lithotropsy.
b) Percutaneous Nephrolithotomy(PCNL)
c) Ureteroscopic stone removal
d) Open (incisional) Surgery

In the present study we are adopting ethylene glycol induced urolithiasis model.

ETHYLENE GLYCOL INDUCED UROLITHIASIS

Ethylene glycol (EG) is a colourless, odourless, viscous dihydroxy alcohol, the exposure of which is toxic to the human body. Ingestion and dermal exposure are the two major routes of exposure to ethylene glycol in which the dermal exposure is unlikely to lead to toxic effects. The ingestion of EG leads to toxic effects. Once EG is ingested it is readily absorbed by the gastrointestinal tract. Distribution is rapid and occurs throughout the body. Peak concentration following ingestion occurs within 1-4 hours. In humans and primates the toxicity of EG is caused by the metabolite rather than the parent molecule. The liver and the kidneys are the major sites of metabolism of EG. Glycolic acid is the primary metabolite.[40]

EG is first oxidised by alcohol dehydrogenase to glycoaldehyde, which is then further metabolised to glycolic acid by mitochondrial aldehyde dehydrogenase

and cytosolic aldehyde oxidase. Glycolic acid is then metabolised to glyoxylic acid by glycolic acid oxidase or lactate dehydrogenase. Glycolic acid oxidase also catalyses the formation of oxalic acid from glyoxylicacid.Glyoxylic acid may be metabolised to malate, formate or glycine. Lactic acid is also formed in the metabolic processes following ethylene glycol exposure. It is the accumulation of these acid products that accounts for much of the toxicity of ethylene glycol. Chelation of aqueous oxalic acid with calcium ions forms insoluble calcium oxalate, which cannot be further metabolised by humans. Excretion of ethylene glycol is primarily in the urine either as the parent molecule, glycolic acid, calcium oxalate or glycine (and its conjugate hippurate). Oxalic acid is excreted in the urine and may give rise to dihydrate and or monohydrate oxalate crystals which may precipitate in the kidney causing nephrotoxicity. Approximately 20% of a dose of ethylene glycol may be excreted unchanged by the kidneys.[41, 42]

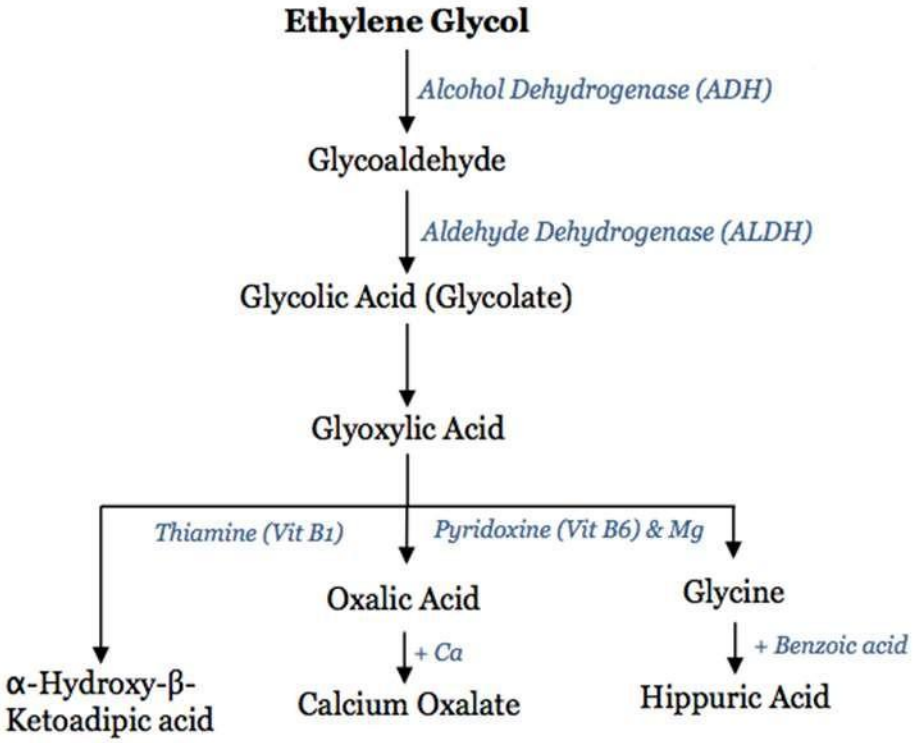


Figure 6: Metabolism of ethylene glycol

CYSTONE[43, 44]

Cystone is a polyherbal preparation mainly used for kidney stones, which is manufactured by Himalaya Herbal healthcare.Cystone also provides support in chronic urinary tract infection and prevents recurrence of stone formation in the kidneys.It also prevents lipid peroxidation and protects the kidney from various nephrotoxic agents. The ingredients are

Shilapushpa _ *Didymocarpus pedicellata*

Pasanabheda – *Bergenia ligulata*

Manjishtha – *Rubia cordifolia*

Nagarmotha – *Cyperus scariosus*

Apamarga – *Achyranthes aspera*

Gojiha – *Onosma bracteatum*

Sahadevi – *Vernonia cinerea*

Main Action

Lithontriptic or lithotropic – dissolves stones in the kidneys

Diuretic

Anti lithiatic – prevents and inhibits stone formation in the kidneys

Reported Activity on Antiurolithiatic Property Of Herbs.

Lulat et al., (2016) investigated the protective effect of Lithocare (LC) (a polyherbal formulation) against ethylene glycol (EG) induced urolithiasis in Wistar rats. Administration of EG in drinking water resulted in hyperoxaluria, hypocalcaemia as well as an increased renal excretion of phosphate. Supplementation with LC significantly reduced the urinary calcium, oxalate, and phosphate excretion dose-dependently. There was a significant reduction in the levels of calcium, oxalate as well as a number of calcium oxalate crystals deposits in the kidney tissue of rats administered with LC in EG-treated rats. There was a significant reduction in creatinine, urea, uric acid, and blood urea

nitrogen when LC was administered in EG-treated rats. From this study, it was concluded that the supplementation of LC protected EG-induced urolithiasis as it reduced the growth of urinary stones. [45]

Anil et al.,(2015) investigated the antiurolithiatic effect of standardized methanolic extract of *Biophytum sensitivum* (MBS) against calcium oxalate urolithiasis in rats. Calcium oxalate urolithiasis was induced in rats by ethylene glycol–ammonium chloride feeding in drinking water. Antiurolithiatic activity of MBS was evaluated in curative and preventive regimen by estimating histological changes in kidney and biochemical changes in urine, serum and kidney homogenate. Cystone (500 mg/kg, p.o.) was used as reference standard drug.Ethylene glycol–ammonium chloride feeding caused an increase in urinary volume, oxalate, total protein, phosphate and uric acid levels, along with a decrease in urinary excretion of calcium, magnesium and citrate. Supplementation with MBS significantly prevented change in the urinary excretion of calcium, oxalate, phosphate, total protein, uric acid, magnesium and citrate and also prevented the elevation of serum creatinine, uric acid and blood urea nitrogen levels. The increased calcium, oxalate,lipid peroxidation and phosphate levels in the kidney of lithiatic control rats were significantly reduced by the MBS supplementation. Histological study revealed minimum damage and less number of calcium oxalate deposits in the kidneys of MBS-treated rats.These results indicated that the MBS reduced and prevented the growth of urinary stones.[46]

Dixit et al., (2014) designed a study to evaluate the effect of Crashcal, a polyherbal formulation in the treatment of renal calculi. The urolithiasis was induced by ethylene glycol. Oxalate, calcium and phosphates were monitored in the urine and kidney. Serum BUN, creatinine and uric acid were also recorded. In-vivo antioxidant parameters such as lipid peroxidation (MDA), glutathione (GSH) and superoxide dismutase (SOD), catalase (CAT), body weight, kidney weight and urine volume were also monitored. In hypercalculi animals, the oxalate, calcium and phosphate excretion grossly increased. However, the increased deposition of stone forming constituents was significantly lowered by Crashcal. It was also observed that Crashcal produced significant decrease in

MDA and increased GSH, SOD and CAT. Thus they reported the antiurolithiatic activity of Crashcal.[47]

Soundararajan et al., (2014) investigated the anti urolithiatic activity of various extract of whole plant of *Ageratum conzoides*Linn on experimentally urolithiasis induced male albino wistar rats. Urolithiasis was induced in animals by using ethylene glycol (5% v/v, 2ml/rat/7days). Experimental induction of hyperoxaluria results in rapid formation of calcium oxalate crystals in the renal tubules of experimental animals. Investigation was done on the basis of estimation of stone forming constituents oxalate, calcium, and phosphate, in kidney and urine. The results proved that the calcuri, ethanolic extracts and ethyl acetate extract significantly lowered the increased levels of oxalate, calcium and phosphate in urine and also significantly reduced their retention in kidney. [48]

Dharmalingam et al., (2014) reported the anti-urolithiatic activity of the aqueous and alcoholic extracts of *Melia azedarach* Linn leaves in calcium oxalate urolithiasis in male albino rats. Lithiasis was induced by oral administration of ethylene glycol (0.75 %v/v) in male albino rats for 28 days. Each of the extract (250 mg/kg) was administered orally on day 0 as a prophylactic regimen and from day 15 as a curative regimen. Regular administration of ethylene glycol caused hyperoxaluria in ethylene glycol-fed animals, leading to increased renal retention and excretion of oxalate, calcium and phosphate. Histopathological study, urine microscopy, serum analysis and biochemical analysis of kidney homogenate were performed. Oxalate and calcium excretion in urine increased respectively, in lithiatic control animals compared to (0.37 ± 0.01 and 1.27 ± 0.12 mg/24 h) for the normal control group. Treatment with aqueous or ethanol extract significantly ($p < 0.01$) reduced the elevated levels of calcium, oxalate and phosphate excretion in urine to 0.79 ± 0.01 and 1.09 ± 0.04 mg/24 h, respectively. Histopathological data for the kidney supported the foregoing results.[49]

Thangarathinam et al.,(2013) reported the effect of Polyherbal Syrup (Aqueous decoction of *Aerva lanata, Astercantha longifolia, Cucumis sativus, Cumimum*

cyminum, Hemides musindicus, Lagenaria siceraria and Tribulus terrestris) against ethylene glycol–induced urolithiasis in male Wistar albino rats . Urolithiasis was induced in rats by administering 0.75% ethylene glycol in drinking water for 28 days and was manifested by high urinary calcium, phosphorus and low urinary volume, pH, magnesium content and high uric acid, creatinine, urea and BUN in serum. Simultaneous administration of Polyherbal syrup (100 and 200 mg/kg/oral) and standard drug hydrochlorothiazide 150µg/kg orally for 28 days along with ethylene glycol (0.75%) reduced urinary calcium, phosphorus and increased urinary volume, pH and magnesium and reduced uric acid, creatinine, urea and BUN in serum. The histopathological studies confirmed the induction of urolithiasis as microcrystal deposition observed in sections of kidney from animals treated with ethylene glycol. This was reduced after treatment with the Polyherbal syrup.[50]

Bhaskar et al.,(2012) evaluated the effect of ethanolic extract of *Nymphaea alba* linn on urolithiatic rats .The urolithiasis was induced by inserting zinc disc (a foreign body) in the urinary bladder followed by supplementing 1% ethylene glycol in drinking water. The reduction in weight of the stones was used as criteria for assessing the preventive or curative regimen. Ethanolic extract of dried leaves of *Nymphaea alba* Linn was administered orally. It was studied by administering two different doses of the plant for prophylactic and curative groups. Oral administration of the *Nymphaea alba* Linn has resulted in significant reduction in the weight of bladder stones compared to the control group.[51]

Lin et al., (2012) aimed to investigate the effects of *Flos catharmi* on calcium oxalate (CaOx) formation in ethylene glycol (EG)-fed rats. For all experimental animals, 24-h urine and blood samples were analyzed at the beginning and end of the experiment. Kidney tissue was histopathologically examined using a polarized light microscope, and crystal deposits were evaluated by a semi-quantitative scoring method; these scores were significantly lower in the FC groups (600 and 1,200 mg/day) than in the placebo group. Thus, they reported the antilithiatic effect of *Flos catharmi*.[52]

Paras et al.,(2012)evaluated the saponin rich fraction prepared from fruits of *Solanum xanthocarpum* (SXS) for antiurolithiatic activity by *in vitro* and *in vivo* studies. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%). Various biochemical parameters in urine, serum and kidney homogenate were analysed after 28 days. Kidneys were also subjected to histopathological analysis. The lithogenic treatment caused polyuria, renal function damage and oxidative stress, manifested as increased malondialdehyde, depleted reduced glutathione and decreased antioxidant enzyme catalase activities of the kidneys, were prevented by simultaneous administration with SXS. [53]

Sathya et al., (2012) reported the antilithiatic activity of the ethanolic extract of roots of *Saccharum spontaneum* Linn.in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%) in adult male wistar albino rats for 28 days. The ionic chemistry of urine was altered by ethylene glycol (EG), which elevated the urinary concentration of crucial ions, viz. calcium, phosphorus and protein thereby contributing to renal stone formation. A significant reduction in the elevated level of these ions in urine of the extract treated animal marked the antiurolithiatic activity of the plant.[54]

Sathish et al., (2010) studied the antilithiatic effect of *Hygrophila spinosa* on ethylene glycol induced lithiasis in male albino rats. The lithiasis was induced to rats by oral consumption of ethylene glycolated water (0.75v/v) for 28 days. Aqueous extract of *H. spinosa* (200mg/kg) was administered orally from 1st day for preventive regimen and from 15th day for curative regimen. The parameters assessed were the calcium, oxalate, inorganic phosphate, protein concentration in urine. The *H.spinosa* significantly ($P<0.01$) reduced the elevated levels of these ions and protein in urine and significantly ($P<0.01$) elevated the urinary concentration ofmagnesium. The elevated serum creatinine levels of lithiatic rats were reduced by prophylactic and curative regimen of extract treatment. Thehistological findings also showed improvement after treatment with the extract. [55]

Yogendr et al., (2009) studied the effect of oral administration of aqueous and alcohol extracts of *Jasminum auriculatum* Vahl (Oleaceae) flowers on calcium

oxalate nephrolithiasis in male albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate.A significant reduction in the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis was observed in the extract treated group. The curative and preventive treatment using aqueous and alcohol extracts also decreased the increased deposition of stone forming constituents in the kidneys of calculogenic rats. The results indicate that the flowers of *J. auriculatum* are endowed with antiurolithiatic activity.[56]

NEPHROTOXICITY

Nephrotoxicity (from Greek: nephros,"kidney") is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. There are various forms of toxicity. It is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. When kidney damage occurs, body unable to rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because increasing number of potent therapeutic drugs. The nephrotoxic effect of most drugs is more profound in patients who already suffer from renal impairment. Some drugs may affect renal function in more than one way.

Nephrotoxic agents

Drugs, diagnostic agents & chemical are well known to be nephrotoxic. The following are some of the important nephrotoxic agents.

A. Heavy metals	:	Mercury, arsenic, lead, bismuth
B. Antineoplastic agents	:	Alkylating agents, Cisplatin, Cyclophosphamide, Antimetabolites, Antitumor antibiotics
C. Antimicrobial agents	:	Tetracycline, Acyclovir, Rifampicin
D. Amino glycosides (Tubular cell toxicity)	:	Gentamicin, Amikacin, Kanamycin, Streptomycin
E. Miscellaneous	:	Non-steroidal anti-inflammatory Ibuprofen, Indomethacin

The nephrotoxins damage specific segment of the nephron to a greater extent than the other segments. The proximal tubule is the most commonly affected, because of the presence of inducible type of microsomal mixed function oxidase (cytochromeP-450) which have been implicated in the toxic activation of various agents. This segment is also rich in glutathione and glutathione metabolizing enzymes. The other common sites which can be affected are renal medulla, distal tubule and Loop of Henle. The renal medulla is affected mainly by polyene antibiotics and cyclosporine and that of distal tubule dysfunction is mainly due to non-steroidal anti-inflammatory agents, cyclosporine, pentamidine, trimethoprim, sulphamethaxozole, amphotericin,amino glycoside antibiotics, lithium, and demeclocycline.The functional manifestations of nephrotoxicity can occur at several levels like tubular function abnormalities such as potassium, magnesium and sodium wasting, concentrating defects and reduction in glomerular filtration. However, there are no ideal clues to the occurrence or localization of tubular cell injury. The nephrotoxins induced changes in the tubule cells may be lethal or sub lethal.

In the present study we are adopting cisplatin induced nephrotoxic model.

CISPLATIN IDUCED NEPHROTOXOCITY

Cisplatin (cis-diamminedichloroplatinum II, CP) is a highly effective antineoplastic DNA alkylating agent used against a diverse spectrum of malignancies [57] Although higher doses of cisplatin are more efficacious for the treatment of cancer, many reversible and irreversible side effects including nephrotoxicity, neurotoxicity, bone marrow toxicity, gastrointestinal toxicity and ototoxicity often limit its utility and therapeutic profile [58,59]. Primary targets of cisplatin in kidney are proximal straight and distal convoluted tubules where it accumulates and promotes cellular damage, by multiple mechanisms including oxidative stress, DNA damage and apoptosis.[60,61]Several lines of evidence suggest the role of ROS in the pathogenesis of nephrotoxicity. Cisplatin induces free radical production causing oxidative renal damage, possibly due to depletion of non-enzymatic and enzymatic antioxidant systems.[62,63]

Light and electron microscopy have shown that the CP-induced injury and necrosis in the rat kidney are predominantly localized in S3 sub segments of

proximal tubular epithelial cells. Morphologically, it is characterized by the loss of microvilli, cellular swelling and condensation of nuclear chromatin .[64] Mitochondrial lysosomes and microsomes are critical CP targets .[65,66] Functional alterations are characterized by change in urine volume, increase in blood urea nitrogen and serum creatinine .[67]There is a continuous search for agents that provide nephroprotection against CP and other platinum drugs. These include antioxidants, modulators of nitric oxide, diuretics, and cytoprotective and apoptotic agents. However, none of these were found to be suitable/safe for clinical use in protecting against CP-induced nephrotoxicity.[68]

Mechanism of action of Cisplatin

It is generally accepted that binding of cisplatin (CDDP) to genomic DNA (gDNA) in the cell nucleus is the main event responsible for its antitumor properties. Once CDDP has been intravenously administered to the patient; it rapidly diffuses into tissues and is highly bound to plasma proteins. Binding of CDDP to plasma proteins is a result of the strong reactivity of platinum against sulphur of thiol groups of amino acids such as cysteine. Hence, near 90% of the platinum in the blood is bound to albumin and other plasma proteins leading to inactivation of a great amount of cisplatin. Loss of the chloride groups from the cisplatin molecule is required before it binds to gDNA. Outside the cell, chloride concentration is around 100 mM. However, within the cell, chloride concentrations range between 2 and 30 mM and cisplatin aquation occurs. Consequently, water molecules replace one or both chloride leaving groups. The result is the formation of the $[Pt(H_2O)Cl(NH_3)_2]+$ and $[Pt(H_2O)_2(NH_3)_2]_2^+$cations. These mono and diaquo species of cisplatin are very reactive towards nucleophile centres of biomolecules because H_2O is a much better leaving group than Cl^-.

Several lines of evidence suggest that mitochondrial DNA, or other mitochondrial targets, are perhaps more important than nuclear DNA damage in mediating cisplatin-induced cell death. Cisplatin is hydrolysed to generate a positively charged metabolite which preferentially accumulates within the negatively charged mitochondria. Thus, the sensitivity of cells to cisplatin appears to correlate with both the density of mitochondria.

Pathophysiology

Cisplatin Uptake and Accumulation in Renal Cells

Uptake of cisplatin is mainly through the organic transporter pathway. The kidney accumulates cisplatin to a greater degree than other organs and is cleared by the kidney by both glomerular filtration and tubular secretion .The cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration suggesting an active accumulation of drug by renal parenchymal cells. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity. [69]

Glomerular filtration, and to a lesser extend secretion are the predominant excretion routes for cisplatin in the rats. No evidence supports the tubular reabsorption. Cisplatin is accumulated by peritubular uptake in both the proximal and distal nephrons. The S3 segment of the proximal tubule accumulates the highest concentration of cisplatin, followed by the distal collecting tubule and the S1 segment in the proximal tubule. In addition to a transporter-mediated process,cisplatin enters the cell through passive diffusion.[70]The contribution of active uptake by a transport system and passive diffusion through the cellular membrane may vary at different sites.

In general, two different membrane transporters are capable of transporting cisplatin into cells: Ctr1 and OCT2. Ctr1 is a copper transporter which was also shown to mediate cisplatin uptake into mammalian cells including ovarian cancer cells. Ctr1 is highly expressed in adult kidney and the protein localizes to the basolateral membrane of the proximal tubule. Down regulation of Ctr1 expression in kidney cells *in vitro* decreased both cisplatin uptake and cytotoxicity, suggesting that Ctr1 is an important cisplatin uptake mechanism in these cells.[71]Transporter mediated uptake is likely the major pathway in renal cells.[72]The organic cation transporter (OCT 2) is the critical transporter for cisplatin uptake in proximal tubules in both animals and humans. Transport mediated by these membrane proteins is polyspecific, electrogenic, voltage-dependent, bi-directional, pH-independent, and Na^+-independent. Three isoforms of OCT have been identified in humans. OCT2 is the main OCT in the kidney, OCT1 is the main isoform of the liver, and OCT3 is widely expressed,

especially in the placenta. Cisplatin is not transported through human OCT1, which may help explain its organ-specific toxicity. Carboplatin and oxaliplatin, the less nephrotoxic analogues of cisplatin, have no interaction with human OCT2.[73]Cimetidine, an organic cation competitor for the transport at human OCT2, reduces cisplatin induced proximal tubule cell apoptosis.[74]Diabetic animals have reduced gene and protein expression of OCT isotypes and are resistant to cisplatin toxicity.

Cisplatin Metabolism

Conversion of cisplatin to nephrotoxic molecules in the proximal tubule cells is required for cell injury. The highest concentration of cisplatin is found in cytosol, mitochondria, nuclei, and microsomes. [75] Cisplatin undergoes metabolic activation in the kidney to a more potent toxin. This process begins with the formation of glutathione conjugates in the circulation, perhaps mediated by glutathione-S-transferase.[76,77]As the glutathione-conjugates pass through the kidney, they are cleaved to cysteinyl-glycine-conjugates by gamma glutamyl transpeptidase (GGT) expressed on the surface of the proximal tubule cells . The cysteinyl-glycine-conjugates are further metabolized to cysteine-conjugates by aminodipeptidases, also expressed on the surface of the proximal tubule cells. The cysteine-conjugates are transported into the proximal tubule cells, where they are further metabolized by cysteine-S-conjugate beta-lyase which is an intracellular enzyme to highly reactive thiols.[78,79]Inhibiton of gamma glutamyl transpeptidase (GGT) and cysteine-S-conjugate beta-lyase enzymes has no effect on the uptake of cisplatin into the kidney but reduces nephrotoxicity. Inhibition of gamma glutamyl transpeptidase activity, however, renders cisplatin inactive as an antitumor drug.[80]

Cisplatin can form monohydrated complexes by hydrolytic reactions. The monohydrated complex is more toxic to the renal cells than cisplatin but it is not kidney specific. The normal low intracellular chloride concentrations promote its formation. Using hypertonic saline to reconstitute cisplatin can decrease the amount of monohydrated complex formed. This approach attenuates nephrotoxicity but may also compromise its antitumor activity.[81]

Cellular Targets of Cisplatin

Platinum compounds are believed to mediate their cytotoxic effects through their interaction with DNA. In an aqueous environment, the chloride ligands of cisplatin are replaced by water molecules generating a positively charged electrophile. This electrophile reacts with nucleophilic sites on intracellular macromolecules to form DNA, RNA and protein adducts.[82]Cisplatin binds to DNA leading to the formation of inter- and intrastrand cross-links, thereby arresting DNA synthesis and replication in rapidly proliferating cells.[82] The finding that cells deficient in DNA repair are more sensitive to cisplatin-induced cell death supports the concept that cisplatin mediates its anti-tumor effects through DNA damage. However, the primacy of nuclear DNA damage as the cause of cisplatin-induced cell death has been challenged. In fact, only a small amount of cellular platinum (<1%) is bound to nuclear DNA and there is a poor correlation between the sensitivity of cells to cisplatin-induced cell death and the extent of DNA platination.[83]

Several lines of evidence suggest that mitochondrial DNA, or other mitochondrial targets, are perhaps more important than nuclear DNA damage in mediating cisplatin-induced cell death.[84]Cisplatin is hydrolysed to generate a positively charged metabolite which preferentially accumulates within the negatively charged mitochondria. Thus, the sensitivity of cells to cisplatin appears to correlate with both the density of mitochondria and the mitochondrial membrane potential [85,86]. This observation may explain the particular sensitivity of the renal proximal tubule to cisplatin toxicity, as this segment exhibits one of the highest densities of mitochondria in the kidney.

Mitochondrial energetics is also disrupted by cisplatin and may contribute to nephrotoxicity. Fatty acids are the major source of energy for the proximal tubule, the primary site of cisplatin kidney injury. Cisplatin inhibits fatty acid oxidation in mouse kidney and in proximal tubule cells in culture through a reduction in PPAR-α mediated expression of genes involved in cellular fatty acid utilization. Cisplatin also affects mitochondrial respiratory complexes and function. [87,88]

Biochemical Changes in the Renal Cell

Cisplatin induces specific gene changes. Genes involved in drug resistance, in cytoskeleton structure and function , in cell adhesion , in apoptosis , in tissue remodeling and in detoxification are upregulated after cisplatin-induced injury. Genesdownregulated by cisplatin include those that localize to the proximal tubules, those that control intracellular calcium homeostasis and those that encode growth factors or their binding proteins. These gene changes are associated with cisplatin damage to proximal tubules, tissue remodeling, and regeneration. [89,90]

Cisplatin-induced nephrotoxicity is mediated by mitogen-activated protein kinase (MAPK) intracellular signalling pathways. The MAPK pathways are a series of parallel cascades of serine/threonine kinases that are activated by diverse extracellular physical and chemical stresses. They regulate cell proliferation, differentiation and survival. The 3 major MAPK pathways terminate in the extracellular regulated kinase (ERK), p38 and Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) enzymes. The ERK pathway is typically activated by extracellular growth factors and has been linked to both cell survival and cell death. ERK and p38 function as an upstream signal stimulating tumor necrosis factor-α (TNF-α) production. ERK also activates caspase 3, which controls apoptosis in renal tubular cells. Phosphorylated-ERK is exclusively localized in the distal nephron; therefore ERK1/2 activation may mediate distal nephron injury P38 activation mediates proximal tubule cells injury.

Intracellular Changes That Damages Renal Cell

The *in vivo* mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, apoptosis, inflammation, and fibrogenesis. High concentrations of cisplatin induce necrosis in proximal tubule cells, whereas lower concentrations induce apoptosis through a caspase-9–dependent pathway [90,91]. The major pathways in cisplatin-induced acute tubular cell injury are shown

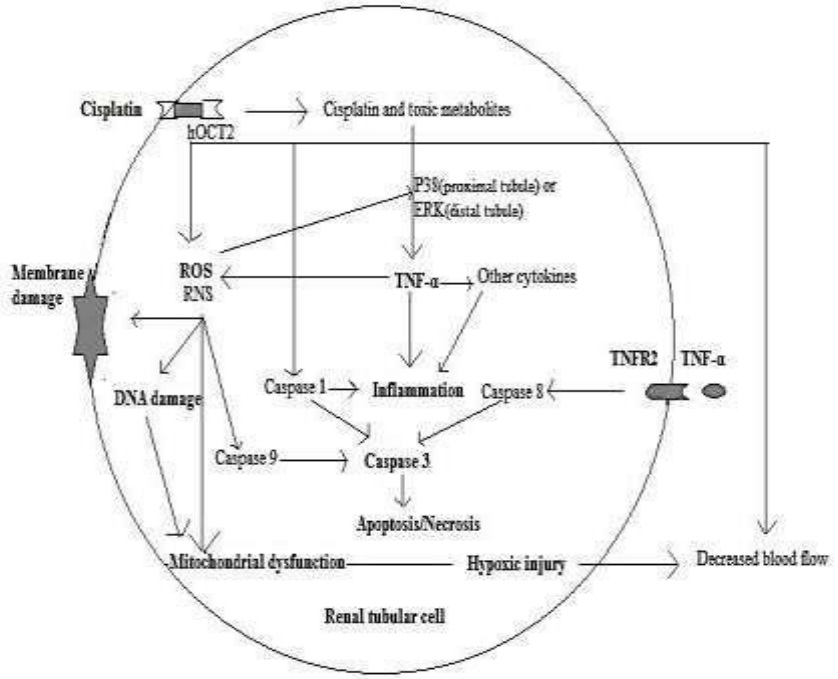


Figure 7 : Major pathways in cisplatin-induced acute tubular cell injury

Role of free radical species

Oxidative stress injury is actively involved in the pathogenesis of cisplatin-induced acute kidney injury. Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins and DNA and destroy their structure. ROS are produced via the xanthine-xanthine oxidase system, mitochondria and NADPH oxidase in cells. In the presence of cisplatin, ROS are produced through all these pathways and are implicated in the pathogenesis of acute cisplatin-induced renal injury. Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production. It increases intracellular calcium level which activates NADPH oxidase and to stimulates ROS production by damaged

mitochondria. Free radicals can also cause mitochondrial dysfunction[92].Reactive nitrogen species have also been studied in cisplatin-induced nephrotoxicity. The renal content of peroxynitrite and nitric oxide is increased in cisplatin treated rats which causes changes in protein structure and function, lipid peroxidation, chemical cleavage of DNA and reduction in cellular defences by oxidation of thiol pools. [93]

Hypoxia and mitochondrial injury

Pathological changes in cisplatin-induced nephrotoxicity occur mainly in the S3 segment of the proximal tubule in the outer stripe of the outer medulla. This zone of the kidney is more susceptible to ischemic insult and injury to this segment occurs in other toxic acute renal failure model [94] . Hypoxic tubules in the outer medulla have been identified by pimonidazole staining in cisplatin nephrotoxicity. Analyses using serial sections indicate that a significant portion of hypoxic cells are proximal tubular cells. Therefore, hypoxia may have an important role in cisplatin-induced nephrotoxicity, and this probably develops during the decreased renal blood observed during the initial phase of cisplatin nephrotoxicity. However, hypoxia inducible factor 1(HIF-1) is activated in the S3 segment of proximal tubules in cisplatin injury *in vivo*. HIF-1 is a transcription factor that mediates cellular responses to hypoxia, including angiogenesis, erythropoiesis and glycolytic adaptation. Dominant negative HIF-1 α-subunit animals have increased susceptibility to cisplatin injury mediated by apoptosis which was associated with the increased release of cytochrome *c*, loss of mitochondrial membrane potential, and increased caspase 9 activities. Therefore, the net effect of hypoxia in cisplatin-induced renal injury is uncertain.95

Apoptosis

The mechanisms of cisplatin-induced nephrotoxicity are complex and involve multiple pathways [96] . The cellular pathways of cisplatin injury to kidney cells have been examined primarily *in vitro* using freshly isolated or cultured renal tubular epithelial cells. *In vitro*, low concentrations of cisplatin preferentially result in apoptotic cell death while at higher concentrations necrosis ensues[97] .*In*

vivo administration of nephrotoxic doses of cisplatin produces a large increase in both necrosis and apoptosis in the kidney. Several apoptotic pathways have been implicated in cisplatin-induced renal epithelial cell death, including the extrinsic pathway activated through death receptors, such as TNF receptors or Fas, the intrinsic mitochondrial pathway and the endoplasmic reticulum stress pathway. Death receptor pathways may be activated by cisplatin include observations that TNFR1 and Fas-deficient renal epithelial cells are resistant to cisplatin-induced cell death that cisplatin increases the activity of caspase 8 and that inhibition of caspase 8 reduces cisplatin-induced cell death *in vitro* [98,99,100].TNF-α stimulates an inflammatory response *in vivo* which exacerbates cisplatin nephrotoxicity[101] .Thus, exposure of renal epithelial cells to cisplatin results in the translocation of Bax to mitochondria, activation of caspase 2, release of cytochome c, AIF,SMAC/Diablo, Omi/HtrA2 and endonuclease G from mitochondria and activation of caspase 9 [102]. Caspases are a family of cell death proteases that play an essential role in the execution phase of apoptosis in cisplatin induced renal tubular epithelial cell death *in vitro* and *in vivo*. Activation of caspases 3, 8 and 9 occur as early as 12 hours after cisplatin treatment of renal epithelial cells *in vitro* and inhibition of caspase activity suppresses cisplatin induced cell death . Both p53 dependent expression of caspases 6 and 7 and p53-independent activation of caspases through Bax/Bak mediated release of cytochrome C contribute to cisplatin induced tubular epithelial cell death[103,104] .The ER stress pathway involves activation of caspase 12 and Ca^{2+} dependent phospholipase A2 and pharmacological inhibition of these enzymes reduces cisplatin-induced apoptosis. Finally, autophagy has recently been shown to participate in cisplatin-induced cell injury.

Cisplatin injury can be ameliorated by free radical scavengers, iron chelators, superoxide dismutase, catalase, selenium and Vitamin E and heme oxygenase-1 induction [104,105]. In summary, cisplatin-induced renal cell death involves multiple pathways including oxidant stress, activation of intrinsic and extrinsic apoptotic cascades and endonucleases (Figure 8). Unfortunately, many of these same pathways contribute to the cytotoxic actions of cisplatin on tumor cells. Therefore, strategies intended to reduce cisplatin renal injury may have the

unintended consequence of reducing the anti-tumor actions of cisplatin. The design of preventive strategies must carefully consider this risk.

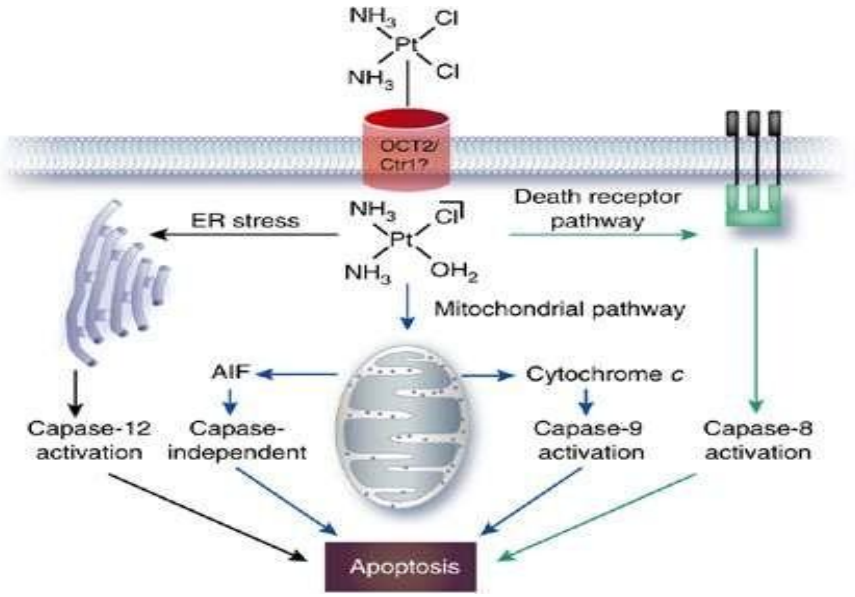


Figure 8 :Apoptotic pathways activated by cisplatin in renal tubular cells.

Inflammation in cisplatin nephrotoxicity

Cisplatin induces a series of inflammatory changes that mediate renal injury. Recent evidences indicate that inflammation has an important role in the pathogenesis of cisplatin-induced renal injury. Cisplatin-induced injury to renal epithelial cells causes release of DAMPs, which activate TLR4. Activation of TLR4 results in the production of a variety of chemokine‟s and cytokines, including TNF-α. These chemokine‟s and cytokines up regulate adhesion molecules and attract inflammatory cells, such as neutrophils and T cells, into the region of injury. Tissue resident dendritic cells act to reduce kidney injury, at least in part by producing the anti-inflammatory cytokine IL-10. Treg cells also reduce kidney injury although the mechanism is still unknown. Dendritic cells also enhance the number or activity of Treg cells. [107,108]

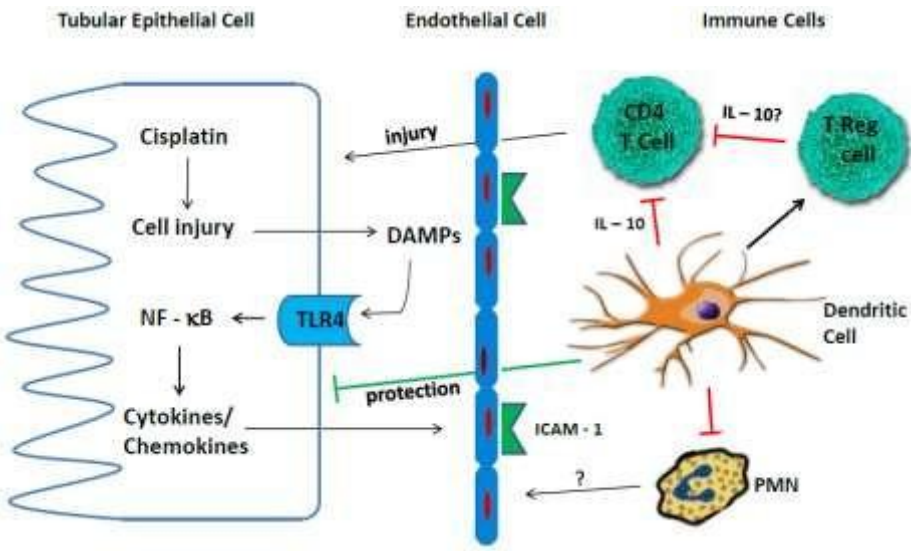


Figure 9 : Immune mechanisms of cisplatin nephrotoxicity.

In summary, cisplatin causes direct tubular injury through multiple mechanisms. Significant interactions among these various pathways may occur during this injury. For example, TNF-α induces apoptosis, produces ROS, and coordinates the activity of a network of cytokines that all contribute to cellular injury. However, it also triggers the expression of inducible nitric oxide synthase, increases the production of nitric oxide and enhances HIF-1 activity in normoxic renal tubule cells, events that could limit injury.How much each pathway and the interactions among these pathways contribute to the cisplatin nephrotoxicity has not been determined.

Reported Activity on Nephroprotective Activity of Herbs.

Tilyek et al., (2016) investigated the nephroprotective effects of *Ribes diacanthum* Pall (RDP) against cisplatin-induced nephrotoxicity. Nephroprotection of RDP was investigated by oral administration of RDP aqueous extract at a daily dose of 40mg/kg for14 consecutive days, starting 7 days prior to cisplatin administration.RDP treatment significantly reduced blood urea nitrogen (BUN) and serum creatinine (Cr) level in cisplatin-administrated mice. Histopathological analysis demonstrated that RDP administration protected cisplatin-induced renal tubular cell apoptosis. Hence reported the nephroprotective effect of *Ribes diacanthum.*[109]

Mohamed et al.,(2016) aimed to determine whether nebivolol possesses a protective effect against cisplatin nephrotoxicity and explore many mechanisms underlying this potential effect. Nephrotoxicity was induced in Wistar rats by a single intraperitoneal injection of cisplatin (6 mg/kg) on day 2. Nebivolol (10 mg/kg) was administered orally for 7 consecutive days. Nebivolol showed a nephroprotective effect as demonstrated by the significant reduction in the elevated levels of serum creatinine and urea as well as renal levels of malondialdehyde, nitric oxide products (nitrite/nitrate), inducible nitric oxide synthase, tumour necrosis factor-alpha, caspase-3, angiotensin II and endothelin-1 with a concurrent increase in renal levels of reduced glutathione and endothelial nitric oxide synthase compared to untreated rats. Histopathological examination confirmed the nephroprotective effect of nebivolol. Pre-treatment with N_{ω}-nitro-L-arginine methyl ester, the non-specific nitric oxide synthase inhibitor partially altered the protection afforded by nebivolol. In conclusion, nebivolol protects rats against cisplatin-induced nephrotoxicity that is most likely through its antioxidant, anti-inflammatory and antiapoptotic effects as well as by abrogation of the augmented angiotensin II and endothelin-1 levels.[110]

Sindhu et al., (2015) investigated the nephroprotective effect of vanilic acid against cis-platin induced nephrotoxicity in male wistar rats. Elevated levels of serum creatinine, bloodurea nitrogen, serum uric acid and reduced antioxidant status were observed as indicatives of nephrotoxicity in cisplatin (7 mg/kg bw) alone administered rats. Animals which are pre-treated with vanillic acid (50 mg/kg and 100 mg/kg) restored the elevated levels of renal function markers and reduced antioxidant status to near normalcy when compared to cis-platin alone treated animals. Cisplatin induced lipid peroxidation was markedly reduced by oral administration of vanillic acid at a high dose. The findings in the present study suggest that vanillic acid is a potential antioxidant that reduce cisplatin nephrotoxicity and can be used as a combinatorial regimen in cancer chemotherapy.[111]

KanchanGaikwad et al., (2013) designed a study to investigate the potential nephroprotective activity of 250mg/kg and 500mg/kg of ethanolic extract of

Adiantum capillus-veneris dried fronds against Cisplatin induced oxidative stress caused in male Wistar rats. Acute nephrotoxicity was induced by i.p. injection of Cisplatin (7 mg/kg of body weight (b.w.)). Administration of ethanol extract at dose level of 500 and 250 mg/kg (b.w.) to Cisplatin intoxicated rats (toxic control) for 14 days attenuated the biochemical and histological signs of nephrotoxicity of Cisplatin in dose-dependent fashion. Ethanol extract at 500 mg/kg decreased the serum level of creatinine and urea as compared to the toxic control group. The ethanol extract of Adiantum capillus-veneris at 500 mg/kg (b.w.) exhibited significant and comparable nephroprotective potential. The statistically (one-way-ANOVAfollowed by Dunnet‟s test) processed results suggested the positive action of *Adiantum capillus-veneris* Cisplatin induced nephropathy.[112]

Sarawoot et al., (2013) investigated the possible protective role of curcumin and α-tocopherol against cisplatin induced nephrotoxicity in rat. Male Wistar rats were divided into five groups. Groups 1 and 2 were intraperitoneally (i.p.) injected with normal saline and cisplatin (20 mg/kg), respectively. Groups 3, 4 and 5 were pre-treated with a single dose of α-tocopherol (250 mg/kg), curcumin (200 mg/kg) and α-tocopherol with curcumin, respectively, for 24 h prior to the administration of cisplatin. After 72 h following injection, specimens were collected. Serum blood urea nitrogen (BUN), creatinine and malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase activities, kidney histopathological study and gene expressions of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and p38 mitogenactivated protein kinase (p38-MAPK) were investigated. Pre-treatment with combined curcumin and α-tocopherol exhibited significantly reduced MDA levels and enhanced activities of SOD and catalase compared with cisplatin-treated group ($p < 0.05$). It also improved BUN as well as creatinine levels and kidney histopathology. Moreover, gene expressions of NADPH oxidase were decreased, whereas p38-MAPK gene expressions were not significant compared with cisplatin-treated group.[113]

Priyadarsini et al.,(2012) evaluated the *Avuri kudineer*[Decoction of *Indigo feratinctoria]* made of indigo leaves AKL, the *Avuri kudineer*made of indigo

root and leaves AKR for nephron protective activity in Cisplatin induced renal damage in rats. Nephrotoxicity was induced in wistar albino rats by intra-peritoneal administration of Cisplatin 5mg/kg. Effect of concurrent administration of AKL and AKRL *Avuri kudineer* at a dose of 500 mg/kg and 1000mg/kg were given for respective animal groups by oral route was determined using serum creatinine and blood urea and change in body weight as indicators of kidney damage. The decoctions significantly decreased the cisplatin induced nephrotoxicity. Remarkable changes were observed in body weight, serum creatinine and urea levels. It was observed that the *Avuri kudineer*[AKRL] significantly protected the kidneys from injury than the *Avuri kudineer*[AKL].[114]

Shelke et al., (2009) Reported the nephroprotective activity of the ethanolic extract of dried fruits of *Pedalium murex* in Cisplatin induced renal damage in rats. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of Cisplatin 5mg/kg. Effect of concurrent administration of the ethanolic extract at a dose of 250 mg/kg given by oral route was determined using serum creatinine and blood urea and change in body weight as indicators of kidney damage. Cystone was used as standard drug. The extract significantly decreased the cisplatin induced nephrotoxicity. Remarkable changes were observed in body weight, serum creatinine and urea levels. It was observed that the ethanolic extract significantly protected the kidneys from injury.[115]

Nitha et al.,(2008) explored the protective effect of the aqueous-ethanol extract of cultured mycelium of *M. esculenta* against cisplatin and gentamicin induced acute renal toxicity in *Swiss albino* mice. Cisplatin and gentamicin when administered induced a marked renal failure, characterized by a significant increase in serum urea and creatinine concentrations. Treatment with various concentration of extract decreased the cisplatin and gentamicin induced increase in serum creatinine and urea levels and also restored the depleted antioxidant defense system. The decreased activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) in the kidneys consequent to cisplatin and gentamicin administration was significantly elevated.[116]Gaurav et al., (2007) evaluated the aqueous extract of *Kalanchoe pinnata* for its protective effects on gentamicin-induced nephrotoxicity in rats.

Effect of concurrent administration of *K. pinnata* leaf extract at a dose of 125 mg/kg/day given by intraperitoneal route was determined using serum and urinary creatinine and blood urea nitrogen as indicators of kidney damage and they observed that the aqueous extract of *K. pinnata* leaves significantly protects rat kidneys from gentamicin-induced histopathological changes. Gentamicin-induced glomerular congestion, peritubular and blood vessel congestion, epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the group receiving the leaf extract of *K. pinnata along* with gentamicin. This extract also normalized the gentamicin-induced increases in urine and plasma creatinine, blood urea and blood urea nitrogen levels .[117]

Annie et al., (2005) studied the ethanolic extract of the roots of *Cassia auriculata* for its nephroprotective activity in cisplatin- and gentamicin-induced renal injury in male albino rats. In the cisplatin model, the extract at doses of 300 and 600 mg/kg body wt. reduced elevated blood urea and serum creatinine and normalized the histopathological changes in the curative regimen. In the gentamicin model, the ethanol extract at a dose of 600 mg/kg body wt. reduced blood urea and serum creatinine effectively in both the curative and the preventive regimen. The extract had a marked nitric oxide free-radical-scavenging effect. The findings suggest that the probable mechanism of nephroprotection by *C. auriculata* against cisplatin- and gentamicin-induced renal injury could be due to its antioxidant and free-radical-scavenging property.[118]

Shirwaikar et al.,(2003) studied the protective effect of various concentrations of ethanolic extract of *Pongami apinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats.The activity was assessed by measuring the loss of body weight ,elevated blood urea, serum creatinine levels and various Histopathological studies.The extract normalized the body weight , raised blood urea and serum creatinine levels.Reversal of cisplatin and gentamicin renal cell damage as induced by tubular necrosis ie, marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination.[119]

PLAN OF WORK

Plan of work

Scores of drugs in common clinical use are capable of inflicting various degrees of damage to the kidney. Similarly, a large number of widely employed chemicals may adversely affect renal tissue as part of their toxic potential. Urolithiasis and nephrotoxicity are two common kidney problems faced by the global population.Urolithiasis is one of the major diseases of the urinary tract and is a major source of morbidity. Stone formation is one of the painful urologic disorders and it is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70-80% in men and 47-60% in women and in economically developed countries the prevalence rate ranged between 4% and 20%.[6, 7].

Nephrotoxicity is another major common kidney problem which occurs when the body is exposed to a drug or toxin. Cisplatin, a platinum compound, is one of the most potent chemotherapeutic agent available to treat a variety of malignancies, including ovarian, lung, head, and neck cancers, as well as testicular and bladder tumors.Unfortunately, cisplatin induces cumulative and dose-dependent nephrotoxicity, which restricts the use of high doses to maximize the therapeutic efficacy. Approximately, one third of patients experience renal dysfunction after treatment with cisplatin

Even though kidney stone and nephrotoxicity was categorized among the most prevalent and widespread disease in the world, till date no guaranteed cure is found for the same. None of the known and available treatments prevent the reoccurrence of kidney stone formation and toxicity produced by cisplatin. Hence the dire need for the herbal formulation appears to be the need of the hour. With the above consideration the study is focused to find a herbal remedy for urolithiasis and nephrotoxicity caused by cisplatin.

METHODOLOGY

METHODOLOGY

A.PLANT MATERIALS

A.1.Collection and identification of the plant materials

The plant *Helicanthes elastica* (Loranthaceae) growing on *Mangifera indica* (Anacardiaceeae) were collected during the month of September –October from ABS Botanical Garden, Kaaripatti, Salem District, Tamilnadu. The mistletoe was authenticated by A.Balasubramaniyam, Executive Director, ABS Botanical Garden, Salem.

A.2. Preparation of methanolic extract of *Helicanthes elastica*

The collected plant materials were washed with water to remove the dirt and other materials and then dried under shade. After confirming the dryness of the plant material, they were grounded with a mechanical grinder to get a coarse powdered plant material and then extracted with methanol using Soxhlet extraction apparatus. The methanol (80%) extract was concentrated using rota vapour under vacuum pressure.

A3) Preliminary phytochemical screening

The qualitative phytochemical screening was performed on the methanolic extract of HE for the qualitative determination of phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, tannins, steroids, terpenoids, carbohydrates, saponins, glycosides, proteins and diterpenes using standard procedures.

Detection of Carbohydrates

a)Molisch's test

Test extract was dissolved in 1ml of distilled water and added 2 drops of alcoholic α-naphthol solution in a test tube and added 2ml of Conc. sulphuric

acid carefully along the sides of the test tube.The formation of violet ring at the junction indicates the presence of carbohydrates.

b)Benedict's Test

Mixed equal volumes of the Benedict‟s reagent and the test extract and heated on a water bath.The formation of orange red precipitate indicates the presence of reducing sugars.

c)Fehling's Test

The extract was hydrolysed with HCl, Neutralized with alkali and heated with Fehling‟s A and B solutions.The formation of red precipitate indicates the presence of reducing sugars

Detection of proteins

a)Biuret test

Treated a small amount of the extract with 4%(w/v) NaOH and 1% (w/v) $CuSO_4$.The formation of violet colour indicate the presence of protein.

b)Ninhydrin test

0.5ml of the extract was treated with 3 drops of Ninhydrin reagent and boiled for few minutes.Formation of blue colour indicate the presence of protein.

Detection of alkaloids.

a)Dragendroff's test

The extract was treated with Dragendroff‟s reagent (solution of potassium bismuth iodide). The formation of red precipitate indicates the presence of alkaloids.

b)Mayer's Test

The extract was treated with Mayer‟s reagent (Potassium mercuric iodide) and the formation of yellow precipitate indicate the presence of alkaloids.

c)Wagner's Test

The extract was treated with Wagner‟s reagent (iodine in potassium iodide) and the formation of brown or reddish brown precipitate indicates the presence of alkaloid.

d)Hager's Test

The extract was treated with Hager‟s reagent (saturated picric acid solution). The formation of yellow coloured precipitate indicate the presence of alkaloids.

Detection of glycosides

a)Keller kiliani test

2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to 0.5 ml of extract diluted with 5 ml water. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer which indicates the presence of cardiac glycosides.

b)Bromine water test

The extract was treated with bromine water.The formation of yellow colour indicates the presence of glycosides.

c)Legal's test

The extract was treated with sodium nitroprusside in pyridine and methanolic alkali.The formation of pink to red blood colour indicates the presence of cardiac glycosides.

Detection of flavavnoids

a) Alkaline reagent test

The extract was treated with few drops of NaOHsolution.The formation of intense yellow colour that becomes colourless on addition of few drop of dilute acid indicates the presence of flavonoids.

b) Lead acetate test

The extract was treated with few drops of lead acetate solution.The formation of yellow colour indicates the presence of flavonoids.

c)Shinoda test

To an alcoholic solution of the extract , few pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid .The appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

Detection of phenolic compounds

a)Ferric chloride test

The extract was treated with few drops of ferric chloride solution. Observed for the formation of brownish green or bluish black colour indicates the presence of phenolic compounds.

Detection of tannins

a)Ferric chloride test

0.5ml of extract was boiled in 10ml of water and filtered. To the filtrate added few drops of 0.1% ferric chloride solution.The formation of brownish green or bluish black colour indicates the presence of phenolic compounds.

b)Gelatin test

To the extract added 1% gelatin solution containing sodium chloride. Observed for the formation of white precipitate which indicates the presence of tannins.

Lead acetate solution was added to the extract.The formation of yellow colour indicates the presence of tannins.

Detection of saponins

a)Foam test

0.5ml of the extract was shaken with 5ml of water and then checked for the foam formation that persist for ten minutes which indicates the presence of saponins.

Detection of steroids and triterpenoids

a)Salkowski's test

2ml of the extract was dissolved in 2ml of chloroform and filtered. The filtrate was then treated with few drops of conc. Sulphuric acid, shaken well and allowed to stand for few minutes.The formation of reddish brown colour indicates the presence of steroids/terpenes.

b)Liebermann Buchard's test

The extract was treated with chloroform and filtered.The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Observed for the formation of brown ring at the junction that indicates the presence of steroids / terpenes.

B.Experimental Animals and Exposure conditions

Healthy Wistar rats of either sex weighing about 180-250g procured from the Government veterinary animal house, Mannuthy, approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were used for the study.The study was approved by the Institutional Animal Ethic Committee of the institute.The rats were housed in standard polypropylene cages with stainless steel top grill and reared at the animal house of Padmavathi College of Pharmacy, Dharmapuri. Paddy husk was used as bedding which was changed at least thrice in a week.Six rats were

housed per cage. All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animas" prepared by the "National Institute of Health". All the procedures were performed in accordance with the Institutional Animal Ethics Committee constituted as per the direction of the by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the ministry of animal welfare division, Government of India, New Delhi, India.

The animals were kept in a clean environment with 12 hours light and 12 hours dark cycles. The temperature was maintained at 25±3^{o}C and the relative humidity was maintained at 45±5%. Animals were fed *ad libitum* with normal laboratory chow standard pellet diet and drinking water in polypropylene bottles with a stainless steel sipper tube throughout the study. The animals were selected randomly. All the animals were acclimatized for 7 days prior to the commencement of the experiment.

C) Instruments

Digital colorimeter (SYSTRONICS)

High speed refrigerated centrifuge (KEMI)

Micro centrifuge (SPINWIN)

Digital balance (SHIMADZU AY220)

UV-Spectrometer (SHIMADZU 1700)

Vertical laminar air flow (KEMI)

BOD incubator (ROTEK)

Hot air oven (KEMI)

Autoclave (KEMI)

D.DrugsAnd Chemicals

Ethylene glycol (Sigma)

Cystone (Himalaya)

Cisplatin

Tween 80

Ethyl ether

E.SelectionOf Dose

Dose selected for the present was based on the acute toxicity studies conducted by M S Rajesh on the metanolic fractions of *Helicanthes elastica.*[15]They reported that neither death nor any observable neuro behavioural effects were observed in the limit test.Hence as stated in OECD guideline no 425,these compounds were classified as globally harmonized system (GHS) category-5 substances.Due to the lack of any observable toxicity at the 2000mg/kg dose, $LD_{50}$was not determined.So two doses were chosen to carryout the present study.The two doses were chosen in such a way that, the first dose was approximately $1/10^{th}$ of the maximum dose administered during acute toxicities(i.e.,$1/10^{th}$ of 2000mg/kg body weight- 200mg/kg body weight) and a high dose which was twice that of $1/10^{th}$ dose i.e.,400mg/kg body weight .

F.EXPERIMENTAL DESIGN

F.1.In- vivo methods-Anti urolithiatic activity

For the assessment of antiurolithiatic activity, two dose levels 200mg/kg and 400mg/kg were chosen. Male Wistar rats were elected from the entire group of animals and grouped in different cages.The dried crude methanolic extracts of whole plant of *Helicanthes elastica* were suspended in 5% tween 80 for oral administration. Antiurolithiatic activity was performed using Ethylene Glycol Induced Urolithiaisis rat models having curative and preventive treatment schedules.

Ethylene Glycol Induced Urolithiasis Model[46,47,48]

The experimental animals were divided into eight groups (n=6) to study the curative and preventive effect of the methanolic extract of *Helicanthes elastica* on ethylene glycol induced urolithiasis in rats.

Group I (Normal control) - Animals were fed with regular rat food and drinking water *ad libitum* from day 1 to 28.

0.75% ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for a period of 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for next 25 days was fed to group II to group VIII for induction of renal calculi.

Group II (Toxic control) - Animals received 0.75% ethylene glycol in drinking water till 28th day.

Preventive treatment

Group III (standard)- Animals received daily single oral dose of standard antiurolithiatic drug Cystone (750mg/kg) first day till 28th day

Group IV - Animals received a single oral dose of the plant extract (200mg/kg body weight p.o) from first day till 28th day.

Group V - Animals received a single oral dose of the plant extract (400mg/kg body weight p.o) from first day till 28th day.

Curative treatment

Group VI (Standard) - Animals received daily single oral dose of standard antiurolithiatic drug Cystone (750mg/kg) from 15th day till 28th day.

Group VII - Animals received a single oral dose of the plant extract (200mg/kg body weight p.o) from 15th day till 28th day.

Group VIII - Animals received a single oral dose of the plant extract (400mg/kg body weight p.o) from 15th day till 28th day.

On day 28 animals of all the groups were kept in metabolic cages and urine samples were collected for 24 hours and analysed for calcium, magnesium and total standard methods. The animals are anaesthetized using ethyl ether and blood samples were collected by cardiac puncturing and serum creatinine, blood urea nitrogen and uric acid were analysed. The urinary output volumes of all groups were also noted. The animals were sacrificed under ethyl ether

anaesthesia and the kidneys are isolated and carried out the Histopathological studies.

F.2.In vivo methods- Nephroprotective activity

Cisplatin induced nephrotoxicity model[114]

The experimental animals were divided into five groups (n=6) to study the curative effect of methanolic extract of *Helicanthes elastica* on cisplatin induced renal toxicity in rats.

Group I (Normal control) – Animals received daily oral dose of the vehicle (CMC 0.6%w/v) from day 1 to day 15(10ml/kg body weight, p.o).

Group II (Toxic control) – Animals were administered with single i.p. dose of cisplatin 5mg/kg body weight on day 1.

Group III (Standard group)-Animals were administered with single i.p dose of cisplatin 5mg/kg body weight on day 1 and Cystone 500mg/kg body weight orally from day 2 till 14.

Group IV – Animals received a single i.p dose of cisplatin 5mg/kg body weight on day 1, followed by daily a single oral dose of the plant extract (200mg/kg body weight, p.o) from day 2 to 14.

Group V – Animals received a single i.p dose of cisplatin 5mg/kg body weight on day 1, followed by daily a single oral dose of the plant extract (400mg/kg body weight, p.o) from day 2 to day 14.

On day 15, animals of all the groups were anasthetised using ethyl ether and the blood samples were collected by cardiac puncture and serum creatinine, total protein and blood urea nitrogen were analysed. The animals are sacrificed at the end of the study and isolated the kidneys and carried out the Histopathological studies on the samples.

G.BIOCHEMICAL ESTIMATION

G.1.MAGNESIUM [120]

Principle

Magnesium ions react with xylidyl blue in an alkaline medium to form purple coloured complex. The intensity of the purple colour is directly proportional to the concentration of magnesium in the specimen.

Mg^{2+} + Xylidyl blue→ Magnesium-Xylidyl blue complex

Calcium is excluded from the reaction by complexing with ethylene glycol tetra acetic acid.

Procedure

Prewarm at room temperature the required amount of reagent before use.

	Sample	**Standard**	**Blank**
	0.01ml	0.01ml	-
Reagent	1.0ml	1.0ml	1.0ml

Incubation

Incubate the assay mixture for 5 minutes at 37^{o}C.After the incubation measure the absorbance of assay mixture against blank at 546nm.Final colour is stable for 30 minutes if not exposed to direct light.

Calculation

Magnesium in mg% = (————————————)

G.2.CALCIUM[120,121]

Principle

Calcium forms a purple coloured complex with cresolphthaleincomplexone in alkaline medium. This complex absorbs light at 575(570-580) nm. The intensity of the colour is directly proportional to the calcium concentration in specimen.

Calcium + cresolphthaleincomplexone→ purple colour

Procedure

Prewarm at room temperature (25-30^{o}C) the required amount of working solution. Perform the assay as given below.

	Sample	**standard**	**Blank**
	0.02ml	0.02ml	-
Working solution	1.0ml	1.0ml	1.0ml

Incubation

Mix and keep the assay mixture for 5 minutes at room temperature(25-30^{o}C).Measure the absorbance against blank at 575nm.(570-580nm).Final colour is stable for one hour if not exposed to direct light.

Calculation

G.3.CREATININE [122]

Principle

Creatinine in alkaline medium reacts with picrate to produce orange colour.This colour absorbs light at 492nm.(490-510nm).The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Creatinine + Picrate→ Orange colour

Procedure

Standard/sample	0.05ml
Working solution	1.0ml

Mix and aspirate.After the initial delay of 30 seconds record the absorbance of the test at an interval of 60seconds at 492nm.Determine the mean change in absorbance and calculate the test results.

Calculation

( )

G.4.TOTAL PROTEIN [123]

Principle

Protein reacts with cupric ions under alkaline pH to produce a colour complex. This colour complex absorbs light at 546nm.(530-570nm).The intensity of the colour is directly proportional to the protein concentration in specimen.

Protein → Blue colour complex

Procedure

Prewarm at room temperature (25-30^{o} C) the required amount of working solution before use.

Perform the assay as given below

	Sample	**Standard**	**Blank**
	0.01ml	0.01ml	-
Working Solution	1.0ml	1.0ml	1.0ml

Incubation

Incubate the assay mixture for 5 minutes at 37^{o}C. After completion of incubation period measure the absorbance of specimen against blank. Final colour is stable for 8hours if not exposed to direct light.

Calculation

(————————————)

G.5.URIC ACID [121,123]

Principle

Uricase converts uric acid into allantoin and hydrogen peroxide.In presence of peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogens to form a red coloured compound, which has maximum absorbance at 510n.(500-530nm).The concentration of the red coloured compound is proportional to the amount of uric acid in specimen.

Uric acid +H_2O→ Allantoin +H_2O_2

H_2O_2 + Phenolic chromogen→ Red colour compound

Procedure

Prewarm at room temperature (25-30^{o}C) the required amount of reagent before use.

Perform the assay as given below

	Sample	**Standard**	**Blank**
	0.025ml	0.025ml	-
Reagent	1.0ml	1.0ml	1.0ml

Incubation

Incubate the assay mixture for 5 minutes at 37^{o} C or 10 minutes at room temperature. After completion of the incubation measure the absorbance of assay mixture against blank at 510nm.Final colour is stable for 30 minutes if not exposed to direct light.

Calculation

RESULTS

RESULTS

1. Preparation of Crude Extract

Crude methanolic extract of the whole plant of *Helicanthes elastica* was prepared by soxhlet extraction with methanol (hydro alcoholic mixture (20:80).

2.Preliminary Phytochemical Screening of Extract

The results of preliminary phytochemical screening of MHE are given below in the table. The MHE extract revealed the presence of carbohydrates, sterols, terpenoids, flavones, phenols, tannins and glycosides.

Table 3: Results of Phytochemical Screening

Sl.No	Test	Inference
1	**Carbohydrates**	
a.	Mollisch‟s test	+
b.	Benedict‟s test	+
c.	Fehling‟s test	+
2	**Proteins and aminoacids**	
a.	Biuret test	–
b.	Ninhydrin test	–
3	**Alkaloids**	
a.	Mayer‟s test	–
b.	Wagner‟s test	–
c.	Hager‟s test	–
d.	Dragendorff‟s test	
4	**Glycosides**	
a.	Keller kiliani test	+
b.	Legal‟s test	+
c.	Bromine water test	+
5	**Flavanoids**	
a.	Alkaline reagent test	+

b.	Lead acetate test	+
c.	Schinoda test	+
6	**Phenols**	
a.	Ferric chloride test	+
7	**Tannins**	
a.	Ferric chloride test	+
b.	Gelatin test	+
c.	Lead acetate test	+
8	**Saponins**	
a.	Foam test	–
9	**Steroids and triterpenoids**	
a.	Salkowski‟s test	+
b.	Liebermann Buchard‟s test	+

Key: + indicates the presence of constituents

- indicates the absence of constituents

ANTIUROLITHIATIC ACTIVITY

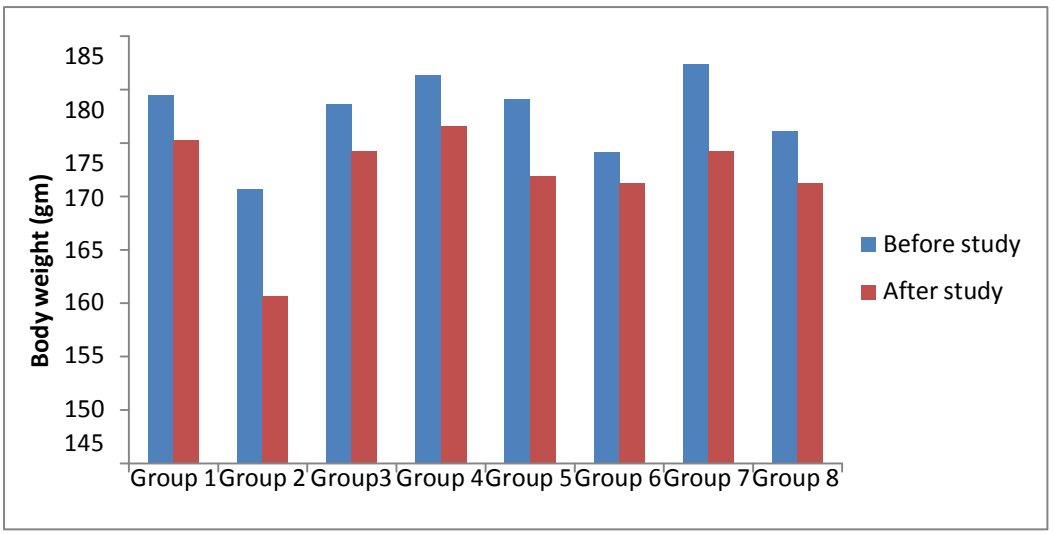
H.1.Determination of body weight

The determination of body weight of all rats in different groups before and after study period shows that, the normal control (Group I) rats increased their body weight. The standard group (Group III and Group IV) maintained their weight with a very little variation. The test group (Group IV) has not much weight loss when compared with the toxic group (Group II) of rats. Results are shown in table 4and graph 1.

Table 4 : Mean ± SEM of body weight determination

GROUPS		BODY WEIGHT Mean ± SEM (g)	
		Before study	After study
Group I	Normal control	179.465±4.3254	175.23±6.2354
Group II	Toxic control	170.66±4.3547	160.67±4.2465
Group III	Preventive standard	178.667±5.2147	174.235±6.3214
Group IV	Preventive 200mg/kg	181.365±5.2454	176.547±1475
Group V	Preventive 400mg/kg	179.166±3.4568	171.894±4.8741
Group VI	Curative standard	174.166±5.2458	171.235±5.4758
Group VII	Curative 200mg/kg	182.356±4.5641	174.23±6.1452
Group VIII	Curative 400mg/kg	176.14±3.847	171.235±3.4574

Effect of MHE on body weights of rats



Graph 1: Body weights of rats before and after study period

Group1: Normal control; Group 2: Toxic control; Group 3: Preventive standard; Group 4:Preventive 200mg/kg; Group 5: Preventive MHE 400mg/kg; Group 6:

Curative standard; Group 7: Curative MHE 200mg/kg; Group8: Curative MHE 400mg/kg

H.2.Urine output determination

Rats were kept in individual metabolic cages for 24 hours for urine collection on 28th day of the study. The normal control rats did not show any significant variation in the urinary output level throughout the experimental period. The urine output was found to increase significantly **(P< 0.001**) by ethylene glycol treated group. Results are shown in table5 and graph 2

Table 5: Statistical analysis of urine output

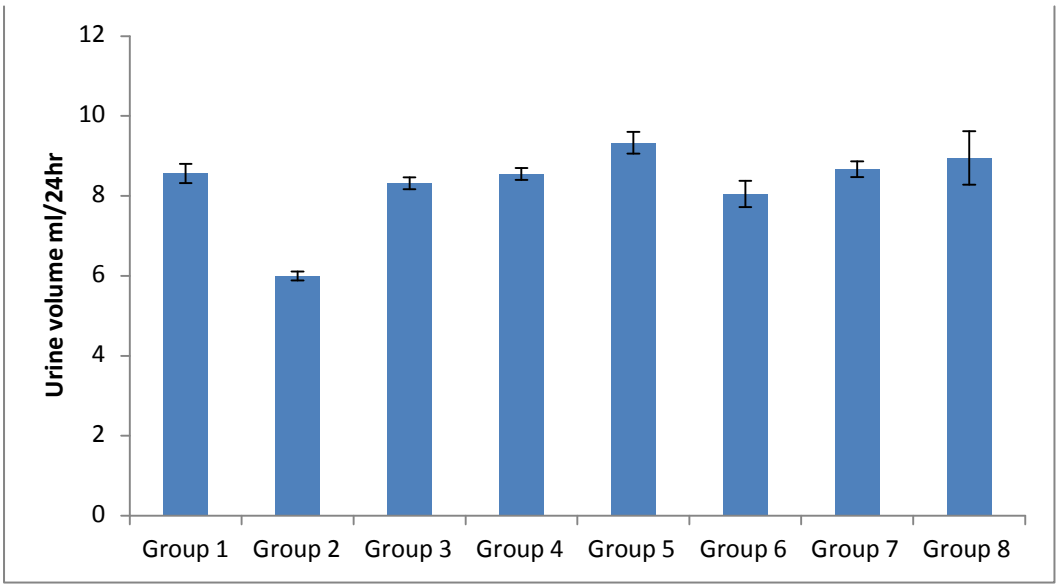
GROUPS		**URINE OUTPUT(ml/24 hr)**
Group I	Normal control	8.56±0.2403
Group II	Toxic control	6.00±0.1125***
Group III	Preventive standard	8.31±0.1492***
Group IV	Preventive MHE 200mg/kg	8.55±0.1477***
Group V	Preventive MHE 400mg/kg	9.33±0.2728***
Group VI	Curative standard	8.05±0.3271***
Group VII	Curative MHE 200 mg/kg	8.66±0.1967***
Group VIII	Curative MHE 400mg/kg	8.89±0.6685***

All the values are expressed as Mean ± SEM (n=6)

P values *** for P<0.001; ** for P<0.01; * for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.

Urine Volume



Graph 2: Effect of MHE on urine volume

H.3.SERUM PARAMETERS

Creatinine, Blood urea nitrogen and uric acid are the important markers for assessment of effect on kidney function.

H.3.1.**Effect on Serum Creatinine**

Serum creatinine level of the control and experimental rats on last day of experiment are shown in Table 6. The serum creatinine level of the normal control rats (Group I) was 0.90 ± 0.1437mg/dl, while it was significantly elevated to 2.50 ± 0.2569mg/dl in animals treated with ethylene glycol (Group II) with a significance level of $P<0.001$.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly reduced the creatinine levels to 0.93 ± 0.2996mg/dl and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly reduced the creatinine levels to 1.06 ± 0.2458mg/dl. The statistical result showed, Group III and Group VI reduced the serum creatinine levels at a significance of $P<0.001$ and $P<0.01$ respectively.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum creatinine level to1.26 ± 0.2044g/dl and 0.96 ± 0.1891mg/dl respectively. The statistical result showed, Group IV and Group V reduced the serum creatinine levels at a significance of P<0.01 and P<0.001 respectively.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly reduced the serum creatinine to 1.40 ± 0.1932mg/dl and 1.16 ± 0.3559 mg/dl respectively when compared with toxic control (Group II).The statistical result showed, Group VI and VIII reduced the serum creatinine levels at a significance of P<0.05 and P<0.01 respectively.

H.3.2. Effect on Serum Blood Urea Nitrogen

Serum blood urea nitrogen level of the control and experimental rats on last day of experiment are shown in Table 6. The serum blood urea nitrogen level of the normal control rats (Group I) was 37.51 ± 1.0514 mg/dl, while it was significantly elevated to 52.53 ± 1.4812mg/dl in animals treated with ethylene glycol (Group II) with a significance of P<0.001.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly reduced the blood urea nitrogen levels to 42.91 ± 1.4639 and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly reduced the blood urea nitrogen levels to 44.86 ± 1.8529mg/dl. The statistical result showed, Group III and Group VI reduced the serum BUN levels at a significance of P<0.001 and P<0.01 respectively.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum blood urea nitrogen level to 48.75 ± 1.6186mg/dl and 43.56 ± 1.1532mg/dl respectively.The statistical result showed, Group V reduced the serum BUN levels at a significance of P<0.001.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly reduced the serum blood urea nitrogen to 48.86 ± 1.4663mg/dl and 45.29 ± 0.8443

mg/dl respectively when compared with toxic control (Group II).The statistical result showed, Group VIII reduced the serum BUN levels at a significance of $P<0.01$.

H.3.3 Effect on Serum Uric acid

Serum uric acid level of the control and experimental rats on last day of experiment are shown in Table 6. The serumuric acid level of the normal control rats (Group I) was 1.88 ± 0.1603mg/dl, while it was significantly elevated to 3.97 ± 0.0227mg/dl in animals treated with ethylene glycol (Group II) with a significance level of $P<0.001$.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly reduced the uric acid levels to 2.52 ± 0.3557mg/dl and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly reduced the uric acid levels to 2.75 ± 0.2366mg/dl. The statistical result showed, Group III and Group VI reduced the serum uric acid levels at a significance of $P<0.01$.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum uric acid level to 3.48 ± 0.1959mg/dl and 2.60 ± 0.2081mg/dl respectively. The statistical result showed, Group V reduced the serum uric acid levels at a significance of $P<0.01$.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly reduced the serum uric acid to 3.75 ± 0.3207mg/dl and 2.94 ± 0.2271 mg/dl respectively when compared with toxic control (Group II).The statistical result showed, Group VIII reduced the serum uric acid level at a significance of $P<0.05$.

Table 6 : Serum parameters of rats used in antiurolithiatic study

Groups	Treatment		Serum Creatinine mg/dl	Serum Blood Urea Nitrogen mg/dl	Uric Acid mg/dl
Group I	Normal control		0.90± 0.143	37.51±1.051	1.88±0.160
Group II	Toxic control		2.50±0.256***	52.53±1.481 ***	3.97±0.227***
Group III	**Preventive treatment**	Standard	0.93±0.299***	42.91±1.463 ***	2.52±0.355**
Group IV		200mg/kg	1.26±0 .204**	48.75±1.618	3.48±0.195
Group V		400mg/kg	0.96± 0.189***	43.56±1.153 ***	2.60±0.208**
Group VI	**Curative treatment**	Standard	1.06±0.245**	44.86±1.852 **	2.75±0.236**
Group VII		200mg/kg	1.40±0.193*	48.86±1.466	3.75±0.320
Group VIII		400mg/kg	1.16±0.355**	45.29±0.844 **	2.94±0.227*

All the values are expressed as Mean ± SEM (n=6)

P values *** for P<0.001; ** for P<0.01; * for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.

I.4.URINE PARAMETERS

I.4.1. Effect on Calcium

Calcium level of the control and experimental rats on last day of experiment are shown in Table 7. The calcium level of the normal control rats (Group I) was 4.92 ± 0.4135mg/dl, while it was significantly elevated to 9.54 ± 0.04839mg/dl in animals treated with ethylene glycol (Group II) with a significance level of $P<0.001$.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly reduced the calcium levels to 5.60 ± 0.3455mg/dl and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly reduced the calcium levels to 5.83 ± 0.5171mg/dl. The statistical result showed, Group III and Group VI reduced the calcium levels at a significance of $P<0.001$.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V respectively) significantly reduced the calcium level to 8.93 ± 0.7202mg/dl and 5.98 ± 0.4755mg/dl respectively. The statistical result showed, Group V reduced the calcium levels at a significance of $P<0.001$.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly reduced the calcium to 8.10 ± 0.6145mg/dl and 6.81 ± 0.8215 mg/dl respectively when compared with toxic control (Group II).The statistical result showed, Group VIII reduced the calcium level at a significance of $P<0.01$.

I.4.2. Effect on Magnesium

Magnesium level of the control and experimental rats on last day of experiment are shown in Table 7. The magnesium level of the normal control rats (Group I) was 2.93± 0.2133mg/dl, while it was significantly decreased to 0.87 ± 0.3022mg/dl in animals treated with ethylene glycol (Group II) with a significance level of P<0.001.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly increased the magnesium levels to 2.21 ± 0.1151mg/dl and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly increased the magnesium levels to 2.19 ± 0.3038mg/dl. The statistical result showed, Group III and Group VI increased the magnesium levels at a significance of P<0.001 andP<0.01 respectively.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V respectively) significantly increased the magnesium level to 1.45 ± 0.1146mg/dl and 2.16 ± 0.8596mg/dl respectively. The statistical result showed, Group V increased the magnesium levels at a significance of P<0.01.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly increased the magnesium to 1.51 ± 0.3674mg/dl and 2.10 ± 0.0573 mg/dl respectively when compared with toxic control (Group II).The statistical result showed,Group VIII increased the magnesium level at a significance of P<0.01.

I.4.3. Effect on Total protein

Total protein level of the control and experimental rats on last day of experiment are shown in Table 7. The total protein level of the normal control rats (Group I) was 2.12 ± 0.1459g/dl, while it was significantly elevated to 5.60 ± 0.1843g/dl in animals treated with ethylene glycol (Group II) with a significance level of $P<0.001$.

In the preventive regimen, the animals which received standard drug, Cystone 750mg/kg(Group III) significantly reduced the total protein levels to 2.60 ± 0.2331g/dl and in Curative regimen , the animals which received standard drug, Cystone 750mg/kg (Group VI) significantly reduced the total protein levels to 2.87 ± 0.2871g/dl. The statistical result showed, Group III and Group VI reduced the total protein levels at a significance of $P<0.001$.

In the preventive regimen, the animals which received MHE at doses of 200 and 400 mg/kg body weight (Group IV and Group V respectively) significantly reduced the total protein level to 4.21 ± 0.2458g/dl and 2.87 ± 0.2271g/dl respectively. The statistical result showed, Group V reduced the total protein levels at a significance of $P<0.001$.

Similarly in curative regimen, the animals which received MHE at doses 200 and 400 mg/kg body weight (Group VII and Group VIII) significantly reduced the total protein to 4.39 ± 0.3232g/dl and 3.15 ± 0.1735 g/dl respectively when compared with toxic control (Group II).The statistical result showed, Group VII and Group VIII reduced the total protein level at a significance of $P<0.01$ and $P<0.001$ respectively.

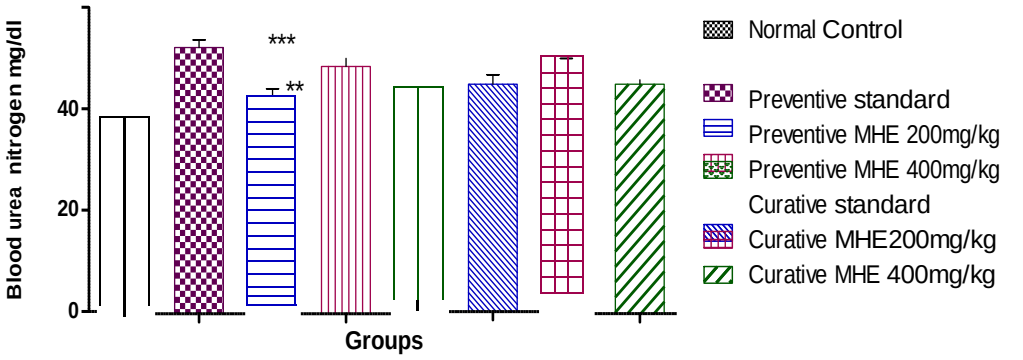
Table 7: Urine parameters of rats used in antiurolithiatic study

Groups	Treatment		Calcium mg/dl	Magnesium mg/dl	Total Protein g/dl
Group I	Normal control		4.92±0.4135	2.93±0.2133	2.12±0.1459
Group II	Toxic control		9.54±0.483***	0.87±0.302***	5.60±0.184***
Group III	Preventive treatment	Standard	5.60±0.345***	2.21±0.115**	2.60±0.233***
Group IV		200mg/kg	8.93±0.720	1.45±0.114	4.21±0.245**
Group V		400mg/kg	5.98±0.475***	2.16±0.659**	2.88±0.227***
Group VI	Curative treatment	Standard	5.83±0.517***	2.19±0.303**	2.87±0.287***
Group VII		200mg/kg	8.10±0.614	1.51±0.367	4.39±0.323**
Group VIII		400mg/kg	6.81±0.821**	2.10±0.057**	3.15±0.173***

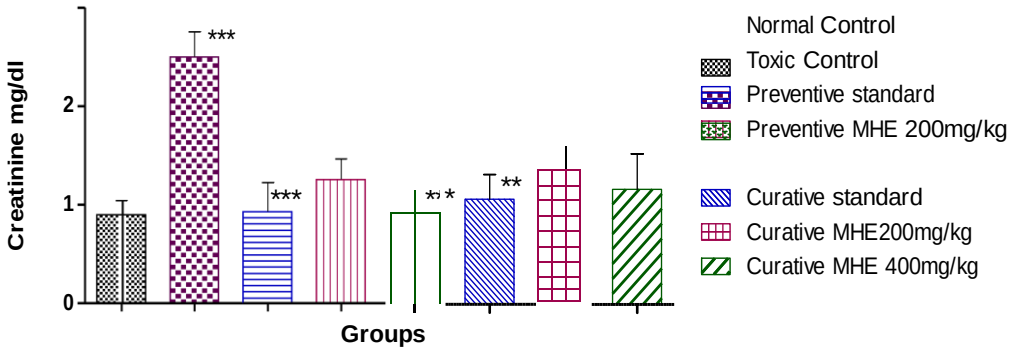
All the values are expressed as Mean ± SEM (n=6)

P values *** for P<0.001; ** for P<0.01; * for P<0.05

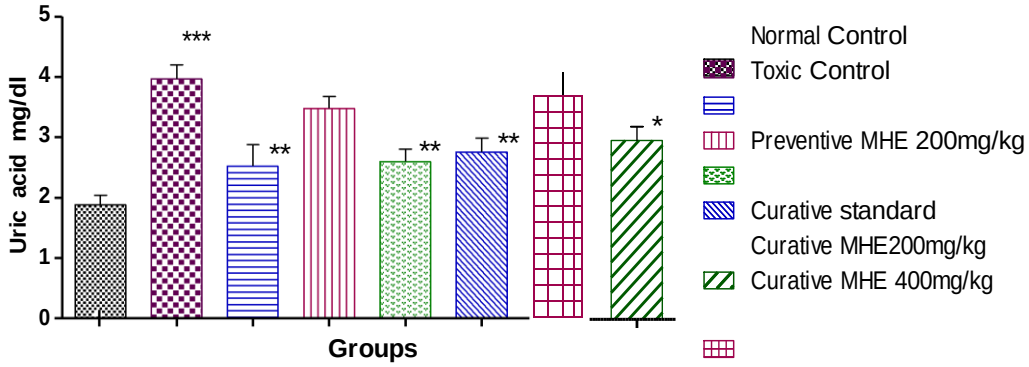
Values of Group II were compared with Group I and those of Group III to V with Group II.



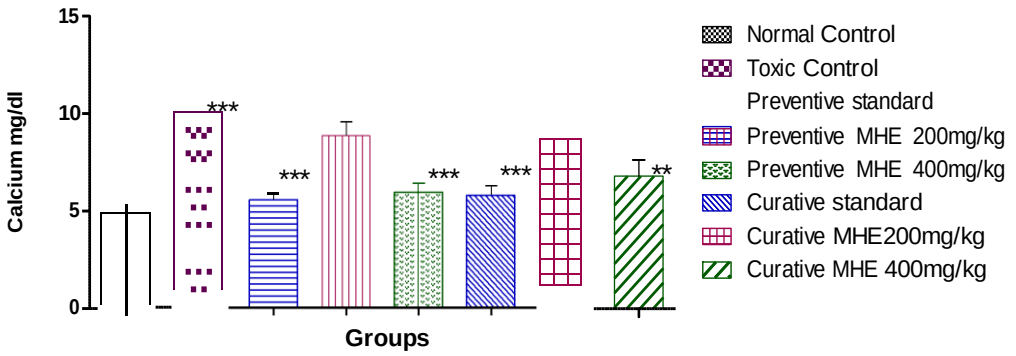
Graph 3:Effect of methanolic extract of *Helicanthes elastica* on serum blood urea nitrogen



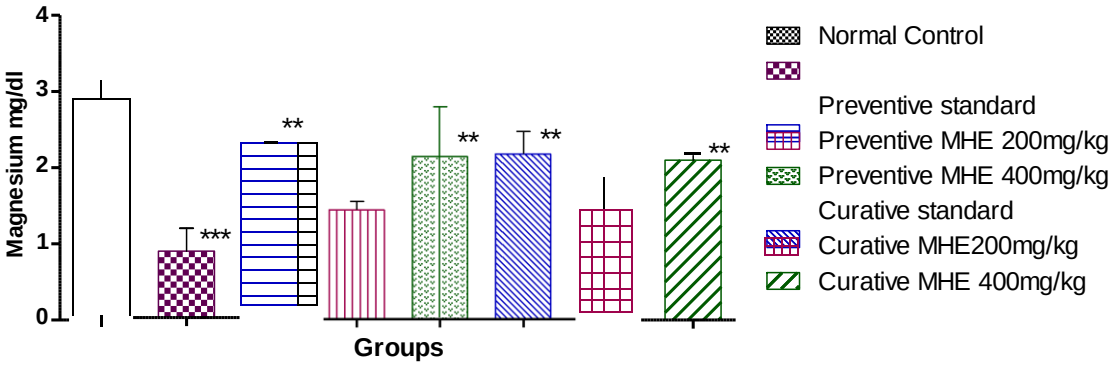
Graph 4:Effect of methanolic extract of *Helicanthes elastica* on serum creatinine



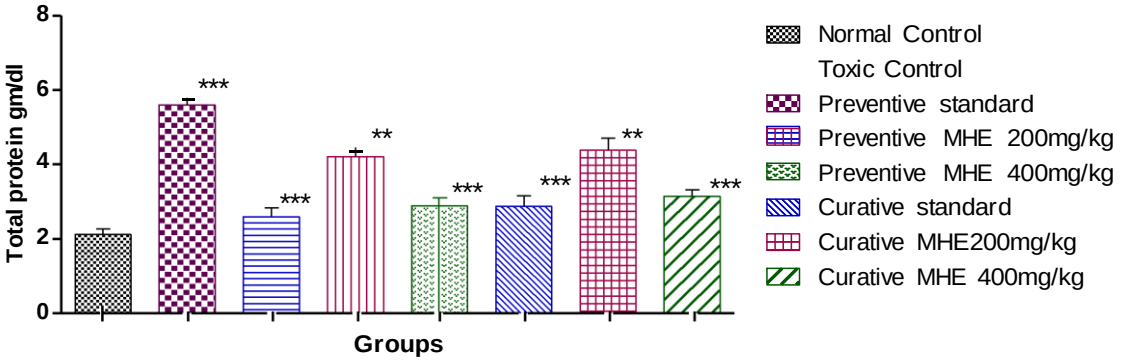
Graph 5:Effect of methanolic extract of *Helicanthes elastica* on serum uric acid.



Graph 6:Effect of methanolic extract of *Helicanthes elastica* on urine calcium.



Graph 7:Effect of methanolic extract of *Helicanthes elastica*on magnesium in urine.



Graph 8:Effect of methanolic extract of *Helicanthes elastica* on total protein in urine.

HISTOPATHOLOGICAL ANALYSIS

Rat kidney histopathology suggested the following observations in the different groups

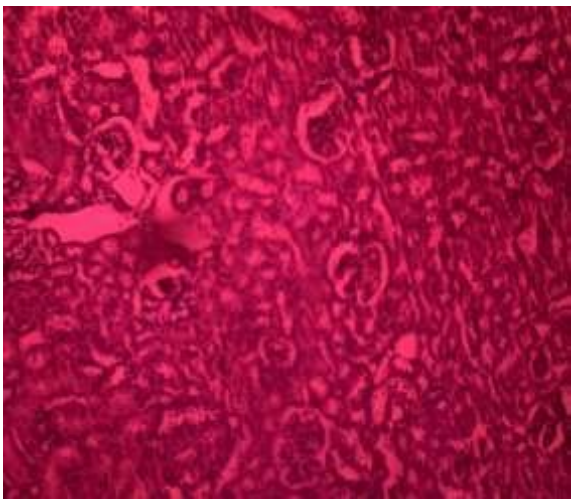


Figure 10a) Normal Control

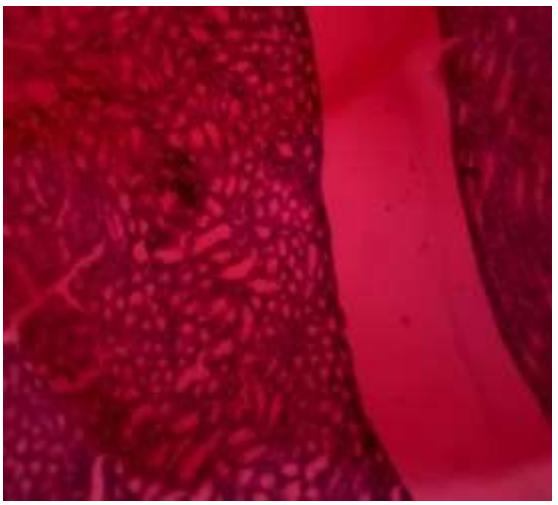


Figure 10b) Toxic Control

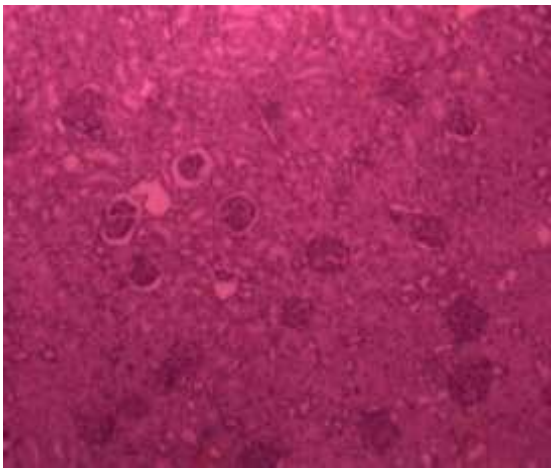


Figure 10c) Preventive Standard

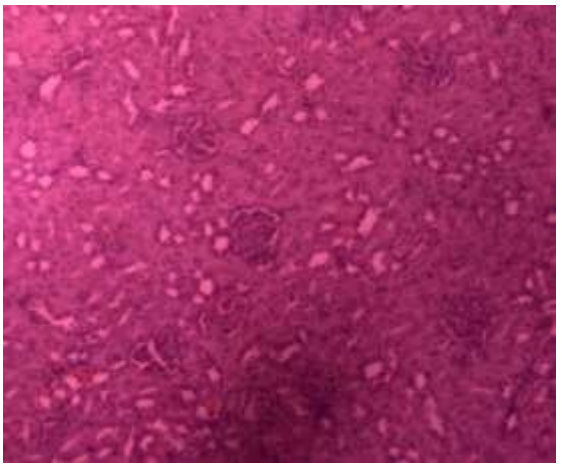


Figure 10d) Curative Standard

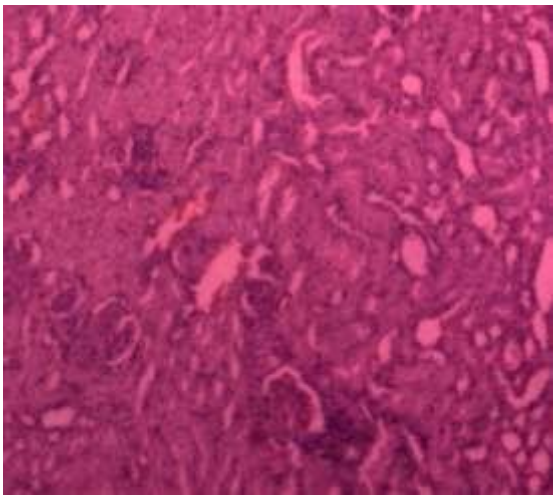


Figure 10e) Preventive MHE 200 mg/kg

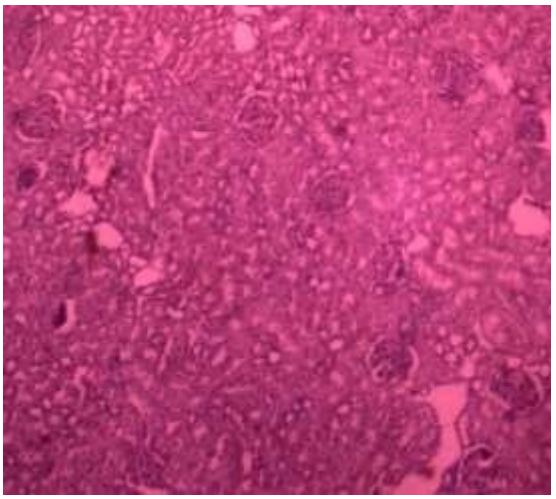


Figure 10f) Preventive MHE 400 mg/kg

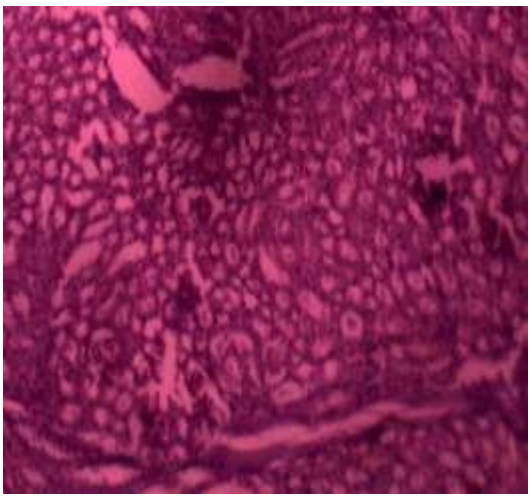


Figure 10g) Curative MHE 200 mg/kg

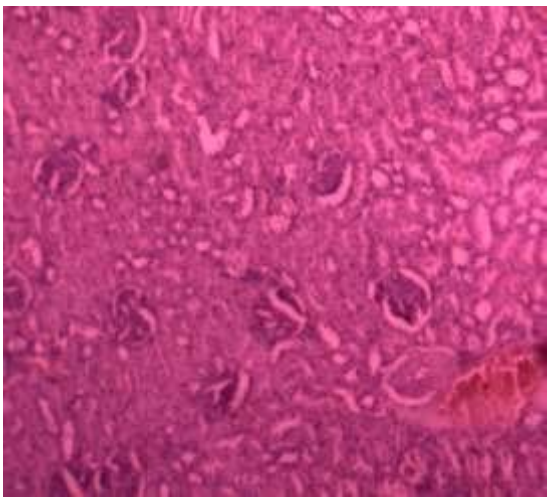


Figure 10h) Curative MHE 400 mg/kg

Figure 10a) Normal kidney: Section shows normal glomeruli and Bowman‟s capsule. Renal tubules are normal .Interstitial tissues appears normal.

Figure 10b) Toxic control: Section of kidney shows glomeruli of varying size. Bowman‟s capsule widened. Renal tubules appears dilated. There are many collections of plasma cells and lymphocytes in interstitial tissue. Foci of calcium oxalate are also seen.

Figure 10c) Preventive standard: Section shows normal glomeruli and Bowman‟s capsule. Afferent and efferent arterioles appear normal. Renal tubules appear slightly dilated and few inflammatory cells in the interstitial tissues.

Figure 10d) Curative standard: Section shows normal glomerulus and Bowman‟s capsule. Afferent and efferent arterioles appears normal. Renal tubules appears normal. A few collections of lymphocytes are seen in interstitial tissues.

Figure 10e) Preventive MHE 200mg/kg: Section shows normal glomerulus and widened Bowman‟s capsule. Renal tubules appears dilated. Scattered inflammatory cells and plasma cells are present in interstitial tissues.

Figure 10f) Preventive MHE 400mg/kg: Section shows normal glomeruli and Bowman‟s capsule. Renal tubules appears normal. Interstitial tissues also appears normal.

Figure 10g) Curative MHE 200mg/kg: Section shows decreased cellularity of glomerulus. Bowman‟s capsule appears slightly dilated. There are foci of calcification. Interstitial tissues showed increased amount of lymphocytes and plasma cells.

Figure 10h) Curative MHE 400mg/kg: Section shows normal glomeruli and Bowman‟s capsule. Afferent and efferent arterioles appears normal. Renal tubules appears normal. Very few collections of lymphocytes in the interstitial tissue.

J.NEPHROPROTECTIVE ACTIVITY

J.1.Effect on percentage change in body weight

.The initial and final body weights of animals of control and experimental groups were taken and percentage change in body weight was calculated and is shown in table 8. Weight of the animals was significantly reduced in cisplatin treated animals (Group II) when compared with normal control (Group I). Decrease in body weight showed significant improvement in the body weight.

Table 8 : Effect of MHEon % change in body weight

Groups	Treatment	% Change in body weight
Group I	Normal Control	-2.18±0.3791
Group II	Toxic control	18.71±2.9696***
Group III	Standard drug	-3.45±0.6511***
Group IV	MHE 200	-8.21±2.0000**
Group V	MHE 400	-4.00±0.6168***

All the values are expressed as Mean ± SEM (n=6)

P values *** forP<0.001; ** for P<0.01; * for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.

J.2.Effect on Serum Urea

Serum urea level of the control and experimental rats on last day of experiment are shown in Table 9. The serum urea level of the normal control rats (Group I) was 29.86± 1.0607 mg/dl, while it was significantly elevated to 59.25± 3.3767mg/dl in animals treated with cisplatin (Group II). In Group III, the animals which received Cystone, the standard drug significantly reduced the serum urea to 35.87 ±3.6114mg/dl. The animals which received plant extract at doses 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum urea to 49.07 ± 2.6755 and 40.04±4.2038mg/dl respectively. The statistical result showed, group IV and Group V (MHE 200mg/kg and

400mgkg respectively) reduced the serum urea levels at a significance of P<0.01 and P<0.05 respectively.

J.3.Effect on serum creatinine

Serum creatinine level of the control and experimental rats on last day of experiment are shown in Table 9. The serum creatinine level of the normal control rats (Group I) was 0.60 ±0.1032 mg/dl, while it was significantly elevated to 3.10 ± .4024 mg/dl in animals treated with cisplatin (Group II). In Group III, the animals which received Cystone,the standard drug significantly reduced the serum creatinine to 1.033 ± 0.3283. The animals which received plant extract at doses 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum creatinine to 2.130 ± 0.2231 and 1.53 ± 0.1115 mg/dl respectively. The statistical result showed, group IV and Group V (MHE 200mg/kg and 400mg/kg respectively) reduced the serum creatinine levels at a significance of P<0.01.

J.4.Effect on levels of Serum Total proteins

Serum total protein level of the control and experimental rats on last day of experiment are shown in Table 9.The serum total protein level of the normal control rats (Group I) was 3.09 ±0.2393g/dl, while it was significantly elevated to 5.60 ± 0.1719g/dl in animals treated with cisplatin (Group II). In Group III, the animals which received Cystone,the standard drug significantly reduced the serum total protein levelto 3.45 ± 0.32886g/dl. The animals which received plant extract at doses 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum total protein to 4.41 ± 0.3818 and 3.93 ± 0.2331g/dl respectively. The statistical result showed, Group V (MHE 400mg/kg) reduced the serum total protein levels at a significance of P<0.01.

J.5.Effect on levels of Serum uric acid

Serum uric acid level of the control and experimental rats on last day of experiment are shown in Table 9.The serum uric acid level of the normal control rats (Group I) was 1.8788 ± 0.1603mg/dl, while it was significantly elevated to 4.03 ± 0.2871mg/dl in animals treated with cisplatin (Group II). In Group III, the animals which received Cystone,the standard drug significantly reduced the serum uric acid levelto 2.39 ± 0.3232mg/dl. The animals which received plant extract at doses 200 and 400 mg/kg body weight (Group IV and Group V) significantly reduced the serum uric acid level to 3.15 ± 0.2424 and 2.4545 ± 0.3718mg/dl respectively. The statistical result showed, Group V (MHE 400mg/kg) reduced the serum uric acid levels at a significance of $P<0.01$.

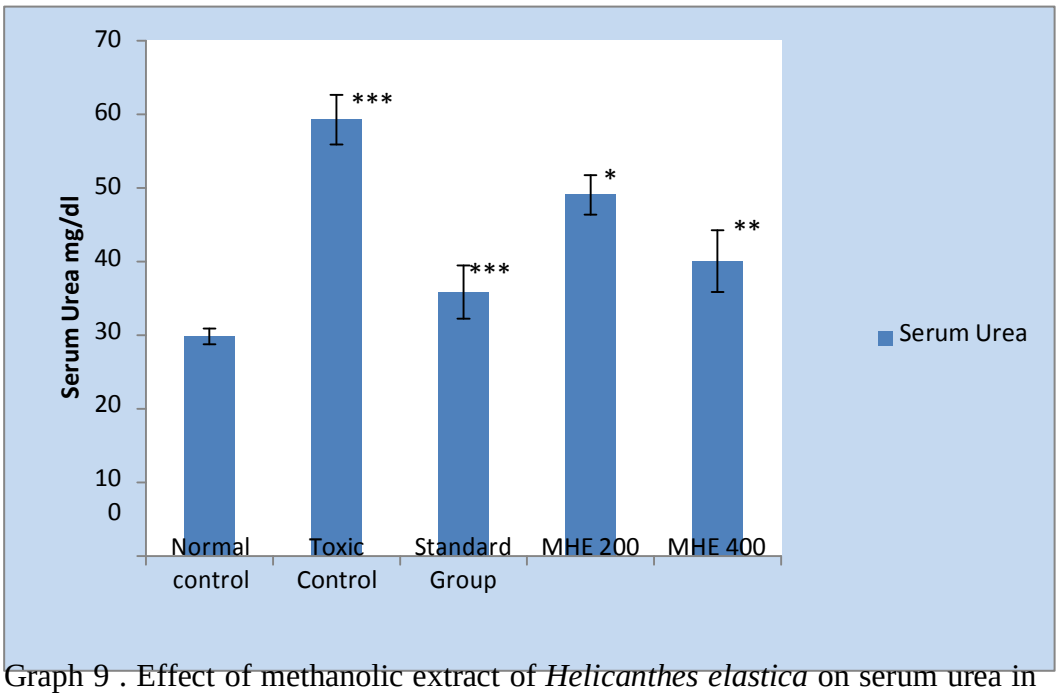
Table 9 . Effect of methanolic extract of *Helicanthes elastica* on Serum urea, Serum creatinine, Serum Total protein and Serum Uric acid in cisplatin induced renal toxicity

Groups	**Treatment**	**Serum urea mg/dl**	**Serum creatinine mg/dl**	**Total protein gm/dl**	**Uric acid mg/dl**
Group I	Normal control	29.86±1.060	0.60±0.103	3.09±0.239	1.87±0.160
Group II	Toxic control	59.25±3.376***	3.10±0.402***	5.60±0.171***	4.03±0.287***
Group III	Standard	35.87±3.611***	1.03±0.328***	3.45±0.328***	2.39±0.323**
Group IV	MHE 200	49.07±2.675*	2.13±0.223**	4.41±0.381	3.15±0.242
Group V	MHE 400	40.04±4.203**	1.53±0.111**	3.93±0.233**	2.45±0.371**

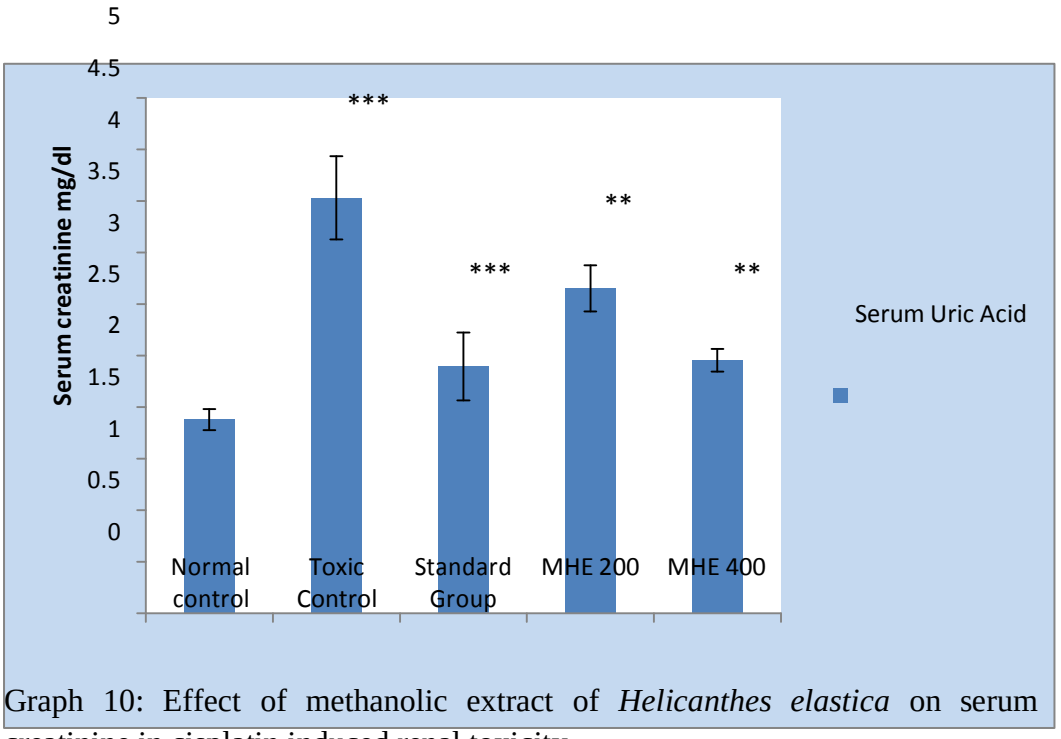
All the values are expressed as Mean ± SEM (n=6)

P values *** forP<0.001; ** for P<0.01; * for P<0.05

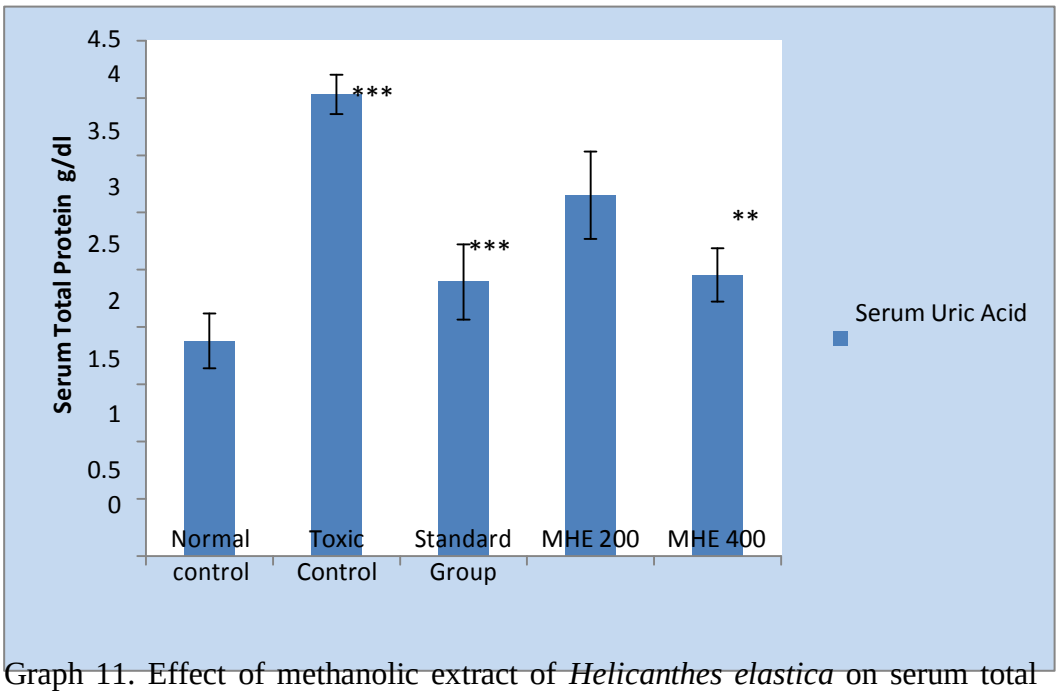
Values of Group II were compared with Group I and those of Group III to V with Group II.



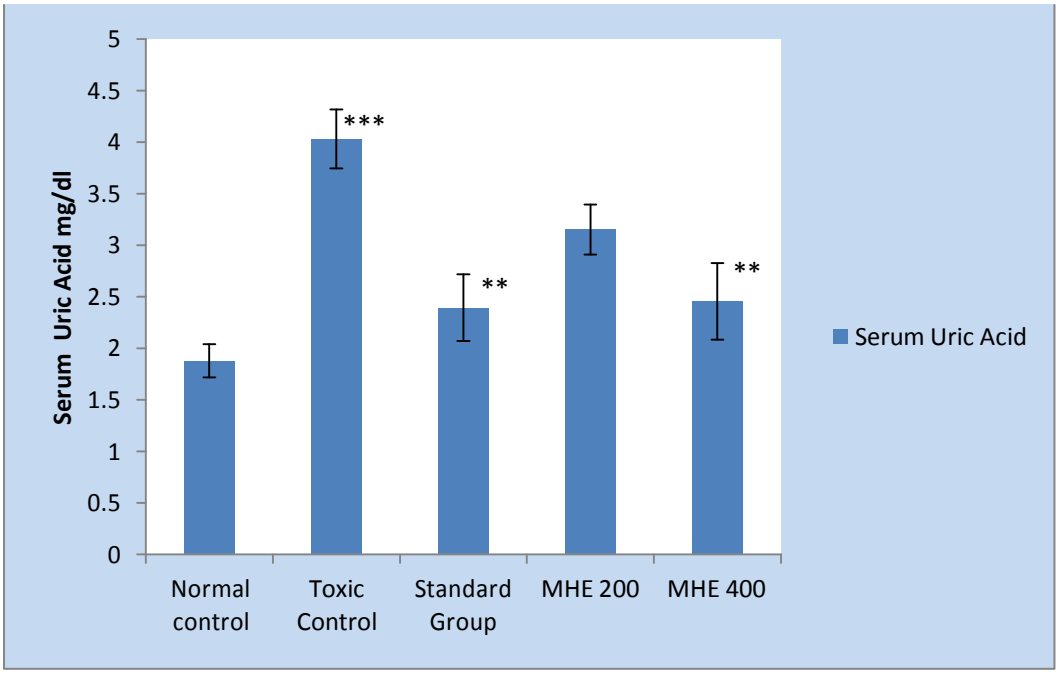
Graph 9 . Effect of methanolic extract of *Helicanthes elastica* on serum urea in cisplatin induced renal toxicity



Graph 10: Effect of methanolic extract of *Helicanthes elastica* on serum creatinine in cisplatin induced renal toxicity



Graph 11. Effect of methanolic extract of *Helicanthes elastica* on serum total protein in cisplatin induced renal toxicity.



Graph 12:Effect of methanolic extract of *Helicanthes elastica* on serum uric acid in cisplatin induced renal toxicity

HISTOPATHOLOGICAL ANALYSIS

Rat kidney histopathology suggested the following observations in different groups

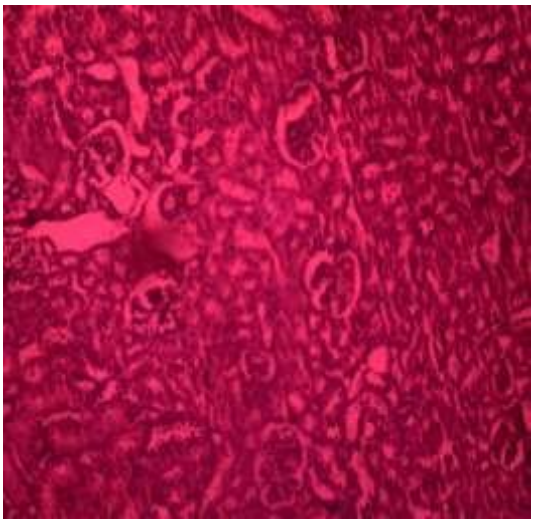


Figure11a) Normal Kidney

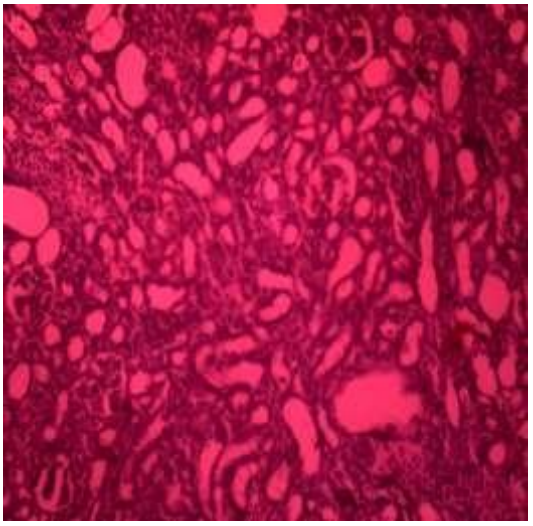


Figure11b) Toxic Kidney (Cisplatin treated)

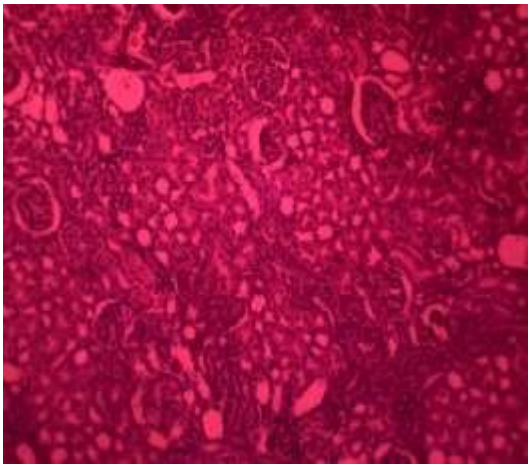


Figure11c) Cisplatin + Standard drug

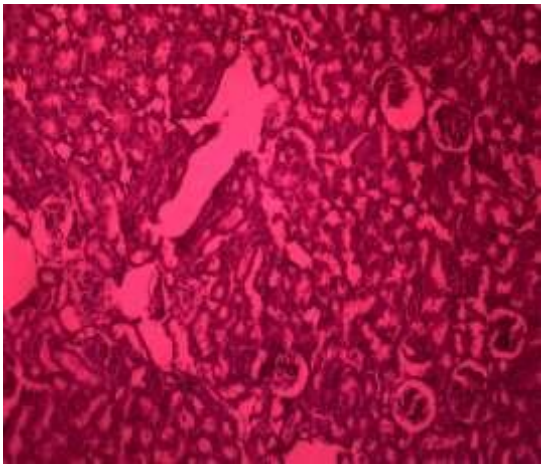


Figure11d) Cisplatin + MHE 200mg /kg

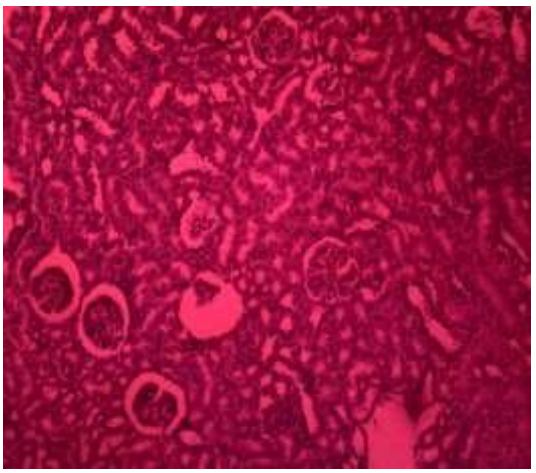


Figure11e) Cisplatin +MHE400mg/kg

Figure 11a) Normal control: Section of kidney shows normal structure. Glomeruli show normal cellularity and vasculature is normal. Bowman‟s capsule appears normal. Renal tubules do not show any specific changes. There are no inflammatory cells or any other abnormal findings.

Figure11b) Toxic control: Section shows smaller glomeruli. Bowman‟s capsule is widened. Glomeruli show diminished cellularity. Interstitial tissues show collection of lymphocytes and plasma cells. Many tubules appear dilated with flattened lining cells.

Figure11c) Cisplatin + Standard drug Cystone: Section shows kidneys with normal glomeruli. Bowman‟s capsule is normal. Afferent and efferent arterioles are normal. There are few collections of lymphocytes. Renal tubules are normal.

Figure 11d) Cisplatin + MHE 200g/kg: Section of kidney shows glomeruli of varying size, some of the glomeruli are normocellular and many glomeruli show decreased cellularity. Bowman‟s capsule is widened. There are small areas of necrosis with collections of lymphocytes and plasma cells, some of the tubules are dilated and lined by flattened cells.

Figure 11e) Cisplatin + MHE 400mg/kg: Section of kidney shows normal glomeruli and Bowman‟s capsule is slightly widened. Renal tubules appears almost normal. Very few collection of lymphocytes and plasma cells are present in the interstitial tissue.

DISCUSSION

DISCUSSION

Antiurolithiatic activity

A number of animal models using rats have been used to induce calcium oxalate urolithiasis. The most reliable and hence commonly employed method is to provide ethylene glycol and ammonium chloride in drinking water to rats. Therefore, in the present study the anti urolithiatic activity of methanolic extract of *Helicanthes elastica* is evaluated using the ethylene glycol induced urolithiasis in rat models.

The biochemical mechanism of ethylene glycol and ammonium chloride induced urolithiasis are related to an increase in the urinary concentration of oxalate. Ethylene glycol is readily absorbed along the intestine and is metabolized in the liver to oxalate leading to hyperoxaluria. Due to the poor solubility of oxalate it precipitates in the urine as calcium oxalate. High oxalate levels and calcium oxalate crystals especially in the nephron damage epithelial cells leading to heterogeneous nucleation followed by causing aggregation of crystals. Furthermore ammonium chloride has been reported to accelerate lithiasis.

Male Wistar rats were selected to induce urolithiasis because the urinary system of male Wistar rats has more resemblance to that of humans.In addition earlier studies have reported that the amount of stone deposition in female rats was significantly less compared to male rats due because of enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation.

Body weights of toxic group rats markedly reduced at the end of the study period due to the decreased feed intake. Body weights of test group rats were also reduced but the rats in the standard group tries to manage their weight loss at the end of study period.

As reported in the previous antiurolithiatic studies [48,49]decrease in urine volume was observed in ethylene glycol induced urolithiatic rats. The methanolic extract of HE treatment also increased urine output but less than the stone induced group. The urine output of the test group was high when compared with

the normal group. This may be due to the diuretic activity of *Helicanthes elastica.*

Consistent with previous reports, [46,47]increased lipid peroxidation has been reported in the kidneys of the rats treated with ethylene glycol. The elevated oxalate concentration has been reported to induce lipid peroxidation and cause renal damage by reacting with the poly unsaturated fatty acids in the cell membrane. This renal damage was indicated by the elevated levels of creatinine, uric acid and BUN in the serum which are the markers of glomerular and tubular damage. The MHE treatment showed to prevent the elevation of serum levels of these markers. This indicates that MHE acts by inhibiting the lipid peroxidation and thereby reduces the extent of tubular dysfunction. The MHE has shown significant restoration indicating better control on the progress of pathology which can be termed as an important characteristic to label it as an ideal therapy.

As reported in some previous studies[46, 47] stone induction by ethylene glycol caused an increase in the concentration of calcium, oxalate and total protein and a decrease in the concentration of magnesium in the urine. In the present study, observed hypercalciuria in ethylene glycol induced urolithic rats favour the nucleation and precipitation of calcium oxalate which leads to subsequent crystal growth. Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone than hypercalciuria. The total protein level was also increased in the lithiatic group. The treatment with MHE significantly reduced the levels of oxalate, calcium and total protein in urine. The potency of the extract may be due to its ability to inhibit some steps of oxalate synthesis or due to the ability to decrease the calcium formation. The treatment with MHE showed to decrease the rate of oxalate, calcium and total protein and thereby reduced the risk of stone formation.

Magnesium is one of the urinary inhibitors of crystallization .Low levels of magnesium are encountered in stone-forming rats as well as in patients with renal stones. Magnesium is reported to form a complex with oxalate and reduce the supersaturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence it reduced the growth and nucleation rate of

calcium oxalate crystals. [125,126] In the present study, the MHE restored the magnesium excretion near to the normal and thus reduced the growth of calcium oxalate crystals. Therefore, the crystallization inhibitory potential of MHE could be a result of increased magnesium content of the urine of MHE treatment rats.

Histopathological studies shows that kidney sections of the calculi induced animals showed accumulation of calcium oxalate deposit which causes marked histological changes such as dilation of the proximal tubules along with the interstitial inflammation. The MHE treatment decreased the calcium oxalate deposits and also reduced the damages to the renal tubules. This may be in part due to the antioxidant effect of the plant extract.

Cystone, a marketed antiurolithiatic composite herbal preparation, is reported to have a protective effect on calcium oxalate urolithiasis. It prevents the accumulation, deposition and supersaturation of the stone forming constituents and thereby inhibits the formation of kidney stones. Cystone also dissolves kidney stones by causing the dissolution of mucin which is responsible to bind the stone particles together. Furthermore it has a diuretic action that flushes out comparatively smaller stones from kidneys[36,126,128] .In the present study, both preventive as well as curative regimens of MHE were effective in reducing the risk of stone formation, decrease in renal tissue injury, reducing the crystal size and thus facilitating easy expulsion and restoring normal kidney architecture. However the preventive effect of MHE was more effective than its curative treatment .This may be due to more potent and anti aggregatory effect of MHE on stone forming constituents than its effect on dissolution of preformed stones suggesting preferable use of MHE in prevention of kidney stones especially in cases of recurrence.

Nephroprotective Activity

Nephrotoxicity is an undesired side effect of chemotherapy in general. Most chemotherapy drugs targets pathways that are essential to dividing cells. Several studies have now documented the importance of reactive oxygen metabolites (ROM) in cisplatin and gentamicin induced renal damage. Nephrotoxicity of the drugs is usually associated with theiraccumulation in renal cortex, dependent upon their affinityto kidneys and on kinetics of drug trapping process. A

minimum dose of cisplatin (5 mg/kg body weight) wassufficient to induce nephrotoxicity in rats. Cisplatin isknown to accumulate in mitochondria of renal epithelialcells and induces ROS primarily by decreasing the activityof antioxidant enzymes and by depleting intracellular concentrations of GSH and also causes the peroxidation ofmembrane lipids. Cisplatin covalently binds to DNAbases and disrupts DNA functions. The cytotoxic action ofthe drug is often thought to be associated with its abilityto bind DNA to form cisplatin-DNA adducts. Cisplatininduced oxidative stress can activate some protein kinases(MAPKs) c-Jun N-terminal kinase (JNK) and p38 whichsensitize the injured cell to apoptosis.

Wistar rats of either sex were chosen for the study. Body weights of toxic group rats markedly reduced at the end of the study period due to the decreased feed intake. Body weights of the test group rats were also reduced but the rats in the standard group tries to manage their weight at the end of the study period.

As reported in various studies [97,129]Cisplatin exposure results in varying degree of lipid peroxidation, inhibition in the activities of antioxidant enzymes and alterations of biochemical parameters and genomic DNA damage in kidneys of rats. It was reported that cisplatin-induced renal toxicity was evidenced by the elevated biochemical markers such as serum urea, serum creatinine, Uric acid and total protein.

Creatinine is a spontaneously generated cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys through glomerular filtration and proximal tubular secretion. There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels increase. This suggests diminished ability of the kidneys to filter these waste products from the blood.[130] Uric acid normally dissolves in the blood, processes through the kidney, and leaves the body in the urine. If the body makes extra uric acid, or if the kidneys cannot clear enough of it, then uric acid levels in the blood will become too high, a condition known as **hyperuricemia**. In the present investigation, serum creatinine level was significantly decreased by MHE at both doses showed its nephroprotective action on cisplatin-induced nephrotoxicity.

Urea is a by-product of metabolism of protein. This waste product is generated in the liver, then filtered from the blood and excreted in the urine by the kidneys. The Urea test measures the amount of urea in the blood sample. High urea levels indicate kidney dysfunction. It was reported that serum concentration of creatinine, urea, uric acid and total proteins depends largely on the glomerular infiltration[131,132]

In the present study, serum urea, BUN, and total protein level were significantly decreased by MHE at both doses showed its nephroprotective action on cisplatin-induced nephrotoxicity. SOD is the first line of defense against free radical induced oxidative stress. It is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radical to hydrogen peroxide. Cisplatin caused a significant decrease in SOD activity. The present investigation showed SOD activity increased by the administration of MHE (400 mg/kg) implicates its antioxidant and nephroprotective activity. GSH is an intracellular reductant and plays a pivotal role in catalysis, metabolism, and transport. It protects cells against peroxides, free radicals, and other toxic compounds . Reduced GSH neutralizes the hydroxyl radical and plays an important role against oxidative stress and inflammatory responses. The nephrotoxicity induced by cisplatin in rats is due to decrease of GSH -S-transferase-γ activity . Cisplatin decreases the GSH content in kidney, indicating oxidative stress. Cisplatin inhibits the activity of antioxidant enzymes (GSH and LPO) in rat kidneys suggests that cisplatin nephrotoxicity results from generation of reactive oxygen species (Aslam et al.,2013;Sakr et al.,2012;Huang et a.,2011). The present study showed GSH content increased by the administration of MHE (400 mg/kg) implicates its antioxidant and nephroprotective activity.

Cystone a polyherbal formulation inhibit the lipid peroxidation by cisplatin in renal corticles at a dose between 500 and 1000mg/kg. Cystone treated animals regained the normal blood urea nitrogen, creatinine and uric acid levels. The main mechanism behind the protective effect against cisplatin is through its lipid peroxidation inhibition.

The histopathology reports also showed that the kidneys of the animals treated with the MHE regained the normal structure. The kidneys of cisplatin treated animals showed large collection of lymphocytes and plasma cells in interstitial tissues. The Bowman‟s capsules were widened and dilated renal tubules. The Cystone standard drug treated animals also regained normal structure.

Phytochemical screening of MHE showed the presence of glycosides, flavanoids, triterpene, steroids, tannin, and phenolic compounds. Anti-oxidant activity of MHE has been already reported. Flavanoids are well known potent antioxidant and free radical scavengers. The whole plant of MHE arerich sources of flavonoids which have been shown to possess several biological properties related to antioxidant mechanism. In the present investigation, the nephroprotective effect showed by MHE may be due to the presence of flavonoids and related compounds.

SUMMARY AND CONCLUSION

CONCLUSION

- The present study was designed to evaluate the antiurolithiatic and nephroprotective activity of methanolic extract of *Helicanthes elastica*.
- Phytochemical analysis of the extract reported positive result for carbohydrates, flavonoids, phenolics, glycosides, tannins, steroids and triterpenoids.
- In the antiurolithiatic study, ethylene glycol was given orally to induce urolithiasis in two different doses for preventive and curative treatment regimen.
- The effect of the plant extract was assessed in terms of serum markers like blood urea nitrogen, uric acid and creatinine and urine markers like calcium, magnesium and total protein. Histopathological studies were also done to support the results.
- Extract treated groups of animals showed significant improvement in serum and urine markers and the kidney damage due to urolithiasis produced by ethylene glycol.
- Based on the results obtained, it can be concluded that methanolic extract of *Helicanthes elastica* possess antiurolithiatic activity against ethylene glycol induced urolithiasis.
- In the nephroprotective study, Cisplatin was given to induce kidney toxicity.
- Extract was orally administered to experimental animals in two different doses.
- The effect of the extract was assessed in terms of serum markers like urea, creatinine, uric acid and total protein. Histopathological studies were also done to support the results.
- Extract treated groups of animals showed significant improvement in serum markers and the kidney damage caused by cisplatin.
- Based on the results obtained, it can be concluded that methanolic extract of *Helicanthes elastica* possess nephroprotective activity against cisplatin induced nephrotoxicity.

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